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Clinical and radiological predictors of neurological toxicity of pontine infusion in diffuse intrinsic pontine glioma

Clinical and radiological predictors of neurological toxicity of pontine infusion in diffuse intrinsic pontine glioma

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Abstract

This thesis describes the treatment on compassionate grounds of 15 children with diffuse intrinsic pontine glioma (DIPG), which is a lethal cancer of middle childhood. Children were treated using chemotherapy infused directly into the pons by chronic, intermittent convection enhanced delivery (CED). The use of an implantable drug delivery system connected to a transcutaneous drug administration port enabled repeated infusions without repeated surgery. Previous experience of pontine infusion in children with DIPG demonstrates that treatment is associated with neurological side-effects. As a new treatment for neurological disease, the cause of these side-effects is unknown. The possible causes can be broadly categorised into those due to the pharmacological action of the drug and the physiological impact of the infusion. This thesis describes how a novel neurological assessment scale was developed, implemented and evaluated to understand the toxicity of pontine infusion. Analysis of the results suggests that the majority of side-effects in brainstem CED are caused by the infusion. Predictive factors of neurological recovery relate to how the infusion is performed. This thesis hypothesises that side-effects are due to localised perfusion deficits arising from high local interstitial pressures within the infused brain. A method of quantitative imaging is described that could lead to a better understanding of this process. On the basis of this work recommendations are made about how a minimally symptomatic infusion could be achieved and how this could be important for the future of CED as a treatment for DIPG.

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Dedication

For children dying with diffuse intrinsic pontine glioma

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Published output

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Preliminary experience of chronic intermittent convection enhanced delivery of carboplatin and valproic acid for the treatment of diffuse intrinsic pontine glioma following radiation therapy. Milo Hollingworth, Cornelia Hurter, Max Wooley, Owen Lewis, Steven Gill, Stergios Zacharoulis. International Symposium on Pediatric Neuro-Oncology, Denver, USA, 2018

The PINE score: a new clinical assessment tool to monitor the neurological signs and symptoms of children with diffuse intrinsic pontine glioma receiving convection enhanced delivery of chemotherapeutics to the brain stem. Milo Hollingworth, Steven Gill, Stergios Zacharoulis. British Paediatric Neurological Association Meeting, London, UK 2018

Author’s declaration

I declare that the work in this dissertation was carried out in accordance with the requirements of the University's *Regulations and Code of Practice for Research Degree Programmes* and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, the work is the candidate's own work. Work done in collaboration with, or with the assistance of, others, is indicated as such. Any views expressed in the dissertation are those of the author.

SIGNED: ..... DATE:.....

Abbreviations

aCSF	artificial Cerebrospinal Fluid
ADC	Apparent Diffusion Co-efficient
ASL	Arterial Spin Labelling
BBB	Blood Brain Barrier
CBF	Cerebral Blood Flow
CED	Convection Enhanced Delivery
CNS	Central Nervous System
CPP	Cerebral Perfusion Pressure
CSF	Cerebrospinal Fluid
CT	Computer Tomography
CTCAE	Common Terminology Criteria for Adverse Events
DIPG	Diffuse Intrinsic Pontine Glioma
DNA	Deoxy-ribonucleic acid
DSC	Dynamic Susceptibility Contrast
DWI	Diffusion Weighted Imaging
FLAIR	Fluid Attenuated Inversion Recovery
GBM	Glioblastoma Multiforme
HDAC	Histone Deacetylase
HDACi	Histone Deacetylase Inhibitors
ICC	Intra-Class Coefficient
ICP	Intracranial Pressure
IQR	Interquartile Range
KPS	Karnofsky's Performance Status
MRI	Magnetic Resonance Imaging
pedNIHSS	Pediatric National Institutes of Health Stroke Scale
PICU	Paediatric Intensive Care Unit
PINEScore	Pontine Infusion Neurological Evaluation Score
SD	Standard Deviation
TABP	Transcutaneous Bone Anchored Port
ΔPINE	Change in PINEScore from pre-infusion baseline

Chapter 1. Introduction

Bypassing the blood brain barrier to improve outcomes for sick children

Children with neurological disease have significantly longer hospital stays and incur double the costs compared to children with diseases outside the nervous system (Moreau et al. 2013). Outcomes for many neurological conditions have failed to improve over the time, with paediatric brain cancer being the obvious example. By comparison, acute lymphoblastic leukaemia was once fatal, but now 90% of those diagnosed in childhood will survive longer than 5 years (Oliver et al. 2014). Yet, brain tumours represent the greatest cancer killer of children, representing the third most common cause of death overall (Ostrom et al. 2014). Around 70% of children are expected to survive over 5 years but outcomes have not significantly changed in the past 20 years (Smith et al. 2010). Developing treatments that access the brain and manipulate underlying pathophysiology remain a major hurdle to improving outcomes for patients with both cancerous and non-cancerous diseases of the central nervous system (CNS).

Normal brain function is dependent on optimal neuronal activity, which in turn is dependent on a tightly controlled microenvironment, which is protected from the chemical fluctuations in the systemic circulation. This is achieved by the blood brain barrier (BBB). As reviewed by Ribatti et al., 2006, the concept of a barrier between the blood and the brain has been around for over 300 years. The first suggestions were documented in the 17th century after it was found that systemic administration of wax and mercury failed to penetrate nervous tissue. 200 years later, Paul Ehrlick in the late 19th century discovered that all organs bar the brain and spinal cord became stained after peritoneal injection of tryptan blue. His student, Edwin

Goldmann, then reversed this experiment by injecting dye into the cerebrospinal fluid - successfully staining the brain and spinal cord but sparing the rest of the body. This clearly demonstrated the compartmentalisation between the body and central nervous system (CNS). Max Lewandowsky in 1900 concluded, from his experiments with cholic acids and sodium ferrous cyanide, that '*the walls of cerebral capillaries hinder the transit of certain compounds'*. This led to an explosion of research marking the beginning of the BBB as we know it. We now understand that barriers protecting the brain go beyond the blood-brain interface and they include:

1. The BBB between the brain and cerebral blood vessels
2. The embryonic CSF-brain barrier between the ventricular system and the extracellular fluid of the brain
3. The blood-cerebral spinal fluid (CSF) barrier between the brain and choroid plexus epithelial cells
4. The arachnoid barrier between the CSF and the dura mater

Nevertheless, the most important barrier in the treatment of neurological disease is BBB, which accounts for the 15-25 m^2 of brain-body interface (Wong et al. 2013). In the 1950s, it was recognised that the lipid molecules were more amenable to transit across the BBB than water-soluble, highly charged or protein-bound molecules (Davson and Smith 1957). In 1967, descriptions of the BBB as a physical barrier became apparent following the introduction of electron microscopy. This allowed the description of the BBB ultra-structure for the first time, including the presence of tight-junctions between brain endothelial cells and the absence of intracellular pinocytic vesicles and fenestrae (Reese and Karnovsky, 1967). A principle component of the BBB is the neurovascular unit, which maintains the BBB via a bi-

directional relationship between brain endothelial cells and surrounding glia, microglia, neurons, pericytes, mast cells and other immune cells (Hawkins and Davis 2005). As reviewed by Banks, 2016, passage of molecules across this barrier can be co-ordinated by:

- immune-active molecules released by immune cells, supportive cells such as glia or astrocytes or by the barrier cells themselves
- trans-endothelial passive diffusion depending on its charge, lipid-solubility, hydrogen bonding and molecular size
- transporters that can actively move molecules as part of an energy dependent process, or facilitate diffusion down a concentration gradient
- glycoprotein binding that can move highly charged molecules during adsorptive transcytosis
- specialised differential functions of the luminal and ad luminal surfaces of the bi-lipid membrane

Pharmacological exploitation of each of these functions represents a promising avenue for drug development. The most obvious pharmacological solution to increasing the concentration of drug in the brain is to increase the concentration administered. High dosing is widely used in clinical practice to treat CNS disease, notably in the treatment of haematological malignancies, but its major limitation is the associated systemic toxicity (Tetef et al., 2000). Other pharmacological solutions of bypassing the BBB remain experimental. Examples include sequestering drugs in the brain by blockade of drug efflux mechanisms (Wong et al., 1993) encapsulation of drugs within transportable nanoparticles (reviewed by Masserini, 2013) and

combination of systemic administration with BBB disruption techniques using focused ultrasound (Lui et al, 2010). However, all these approaches still require systemic exposure to the drug and/or the drug vehicle and depend on efficient penetration of the BBB, which remains a highly sophisticated guardian of the brain-blood interface.

Another approach to access the brain more effectively is to change the route of administration and this can be achieved in a variety of ways. Intra-nasal delivery of insulin has been used to enhance memory function in patients with Alzheimer's disease (Reger et al., 2000). This route exploits the connections between the intra-nasal cavity with the cerebrum and brain stem via the olfactory and trigeminal nerves (reviewed by Crowe et al, 2018). Intra-thecal administration is a well-recognised route used in anaesthesia, chronic pain management, spasticity and delivery of chemotherapy for some cancers (Deer, 2001; Penn, 1985; Ruggerio, 2001). However, the use of intra-thecal delivery is constrained by practical limitations of accessing the CSF, risks of introducing highly neurotoxic drugs to the CNS and the inability to treat deep-seated intraparenchymal disease (Blayney et al., 1995; Larson et al., 1971; Poplack et al., 1980). Drug-impregnated media can also be applied locally at surgery. Wafers impregnated with 1,3-bis (2-chloroethyl)-1-nitrosourea (Gliadel®) can be used to line the resection cavity following glioblastoma multiforme (GBM) surgery and have been shown to increase survival from 11.6 months to 13.9 months (Westphal et al., 2003). Similarly, mouldable polymers loaded with etoposide and methotrexate have also been used to control local disease within the tumour bed (Rahman et al., 2013). Intra-arterial drugs can be used and have the benefit of not requiring surgical access. It has been used in a variety of diseases including brain

cancer, large vessel stroke and delayed cerebral ischaemia following subarachnoid haemorrhage (Joshi et al., 2015; Furlan et al., 1999; Hollingworth et al, 2015). It is performed by cannulation of the carotid or vertebral arteries via peripheral arteries such as the femoral or radial artery. Intra-arterial drug embolization aims to exploit the brain's capillary networks to reach the target site where diffusion distances are relatively short (Joshi et al., 2008). However, such therapy has failed to reach mainstream clinical practice likely owing to concerns about cerebrovascular injury, the absence of clinical evidence to demonstrate superiority over intra-venous delivery and the expense and availability of endovascular services (Joshi et al., 2008).

A limitation of these treatments is that after bypassing the BBB ongoing drug distribution depends on passive diffusion (Lieberman et al. 1995). This can result in small heterogeneous treatment volumes and suboptimal local disease control (Barua et al., 2014). Homogenous drug distribution through large brain volumes can be achieved using convection enhanced delivery (CED) (Bobo et al., 1994). CED refers to intra-parenchymal pressure-driven infusion that establishes a pressure gradient at the tip of an intra-parenchymal catheter; this creates a convection current within the brain interstitium. Drug then displaces extra-cellular fluid by bulk flow through a volume of distribution several times greater than the volume of infusion, which enables delivery of drug through clinically-relevant brain volumes (Bienneman.et al., 2012).

CED has been widely used pre-clinically since the early 1990s (Bobo, 1994) but its failure to translate into clinical practice is dependent on several factors that determine therapeutic efficacy. These include characteristics of the drug and the

performance of the drug delivery device itself, which in turn is dependent on the design of the drug delivery system and its optimal surgical implantation (Lewis et al., 2016).

CED aims to displace extra-cellular fluid - consisting of dissolved anions, cations and extracellular matrix proteins - and replacing it with the therapeutic agent of choice. This model of drug delivery is subject to different principles that govern conventional drug design. In conventional drug design, once a drug has been found to act on a validated target, assays are used to optimise the drug for clinical application. Key tests include measurement of lipophilicity to ensure movement across membranes, metabolic stability to predict liver clearance, interaction with key enzymes such as CYP450 and intestinal absorption (Hughes et al., 2011). If drugs were optimised this way for delivery by CED, highly lipophilic drug would be rapidly cleared from the brain and the direct administration and minimal systemic exposure would render considerations regarding absorption, first and second pass metabolism and CYP450 enzyme interference almost irrelevant.

The ideal drug for CED should be water-soluble, freely distribute in the brain interstitium, maintain a long tissue half-life and should be non-toxic to normal brain parenchyma (Barua et al., 2012). Before drugs are infused into the brain, the drug must be dissolved in media that is non-toxic, which can be fully resorbed to prevent permanent damage to the interstitium. The drug and its excipients must also remain in solution at physiological pH and temperature to prevent precipitation of drug within the interstitial space, which could cause cellular damage and impair of drug distribution. The drug must be able to flow freely within the interstitium to maximise the volume of distribution, which is influenced by several factors. Positively charged

drug molecules bind to cellular membranes impeding drug distribution and so using neutral or negatively charged drug molecules are best suited to administration by CED (Saito et al. 2006; Kikuchi et al. 2008). Increasing infusate viscosity can help to increase the efficiency of distribution (Perlstein et al., 2008). Molecules that have affinity for extracellular matrix proteins also limit distribution. For example, adenoviral vectors have heparin binding regions and hence co-infusion with heparin helps to saturate extra-cellular matrix heparin sulphate molecules and aid distribution (Hamilton et al. 2001). These factors must all be considered to maximise the efficacy of drug distribution in CED.

The drug itself must also be non-toxic to underlying normal parenchyma. This can be problematic in the setting of oncology whereby the infusates are intended to kill cancerous cells and can also damage underlying brain and consequently limit the volume of infusion. Vincristine, for example, is highly neurotoxic and lethal following intrathecal administration despite being an effective anti-cancer agent when delivered systemically (Manellis et al., 1982). Therefore, drugs delivered by CED must distribute efficiently, avoid rapid clearance from the CNS and be therapeutically efficacious without causing irreversible damage to the underlying parenchyma. Hence, extensive preclinical testing of drug stability, tissue half-life, distribution characteristics and toxicity profiles in clinically relevant preclinical models must be performed before drugs can be administered by CED in humans.

Controlling drug distribution is essential to achieve therapeutic efficacy. In CED, regardless of how ideal the drug characteristics may be, any brain volume outside the volume of distribution cannot be feasibly treated. In the PRECISE study (Kunwar et al., 2013), 192 patients with recurrent GBM were randomised to receive IL13-

PE38QQR administered by CED using between 2 and 4 intraparenchymal catheters. There was no improvement in overall survival compared to patients treated with Giadel ® wafers. A reason for this failure has been attributed to inadequate coverage of the target volume (Sampson et al., 2010). Achieving adequate coverage within the target volume is undermined by reflux/back flow along the catheter trajectory away from the catheter tip. This phenomenon can result in variable pressure gradients and thereby reducing the efficiency of CED (Bobo et al., 1994). Factors that are understood to increase catheter reflux are:

- Large catheters, which increase the surface area and reduce resistance for drug reflux (Morrison et al. 1999, Chen et al. 1999, White et al. 2011)
- High infusion rates (Chen et al. 1999)
- Local tissue trauma (White et al. 2011)
- Slower catheter insertion times (Casanova et al., 2014)

Various catheter designs have been developed to control reflux (Figure 1.1), reviewed by Lewis et al., 2016. Step-design catheters, which are tapered toward the end, demonstrate superior reflux control. The exact mechanism behind this is unknown. The recessed-step catheter design, used by the Function Neurosurgery Group, is a further iteration of this and consists of a catheter housed within an inner and outer guide tube (Figure 1.1). The inner guide tube is recessed within the outer guide tube and is thought to control reflux by creation of a tissue seal at the interface of the catheter and guide tube (Gill et al., 2011). This catheter design has been used to deliver drugs to patients with Diffuse Intrinsic Pontine Glioma (DIPG) (Barua et al., 2013), GBM (Barua et al., 2016) and Parkinson's Disease (Whone et al., 2019).

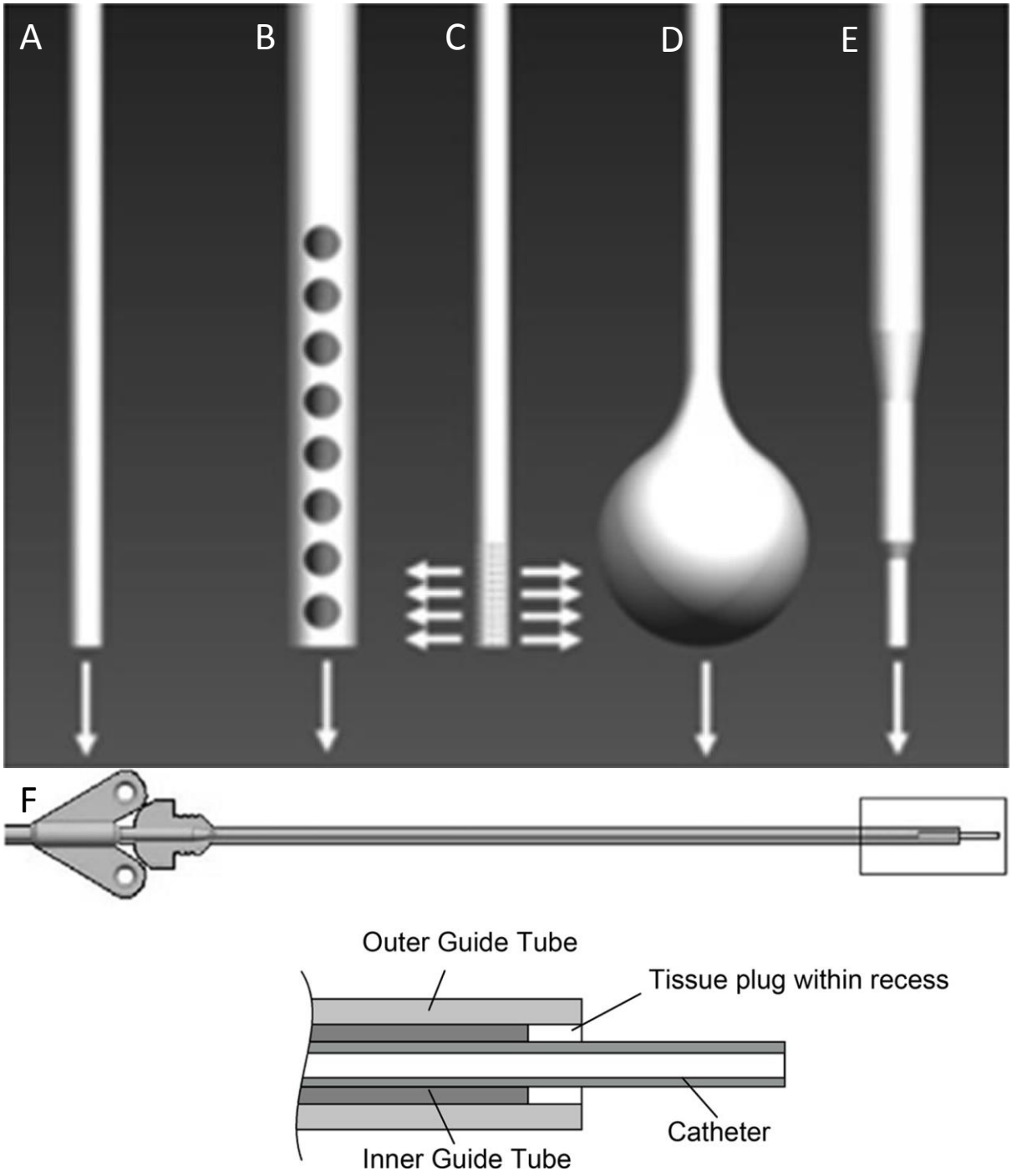

Figure 1.1. Convection enhanced delivery catheter designs. End Port Cannula (A), Multi-Port Cannula (B), Porous Tipped Catheters (C), Balloon Tipped Catheters (D) and Stepped Profile Catheters (E). A recessed stepped catheter design used by the Functional Neurosurgery Group, University Bristol (F). Adapted from Lewis et al., 2016.

Diffuse Intrinsic Pontine Glioma and Convection Enhanced Delivery

DIPG is a rare and lethal disease of middle childhood. The median survival is 11.1 months whilst progression free survival is 6 months (Veldhuijzen van Zanten et al., 2017). Patients typically present with a “classical triad” of ataxia, cranial neuropathy and long tract signs (Johung and Monje, 2017). Initial diagnosis involves magnetic resonance imaging, at which point parents are given a terminal diagnosis and recommendations for treatment and palliative care should be made simultaneously (Veldhuijzen van Zanten et al., 2017). Radiotherapy is the mainstay of treatment, which is given upfront. There is no evidence to suggest survival is better with high dose radiotherapy (78Gy) versus conventional doses (54 Gy) (Packer et al., 1994). Indeed, there is evidence to suggest that hypofractionated doses (34 Gy) achieve similar outcomes (Zaghloul et al., 2014). In some cases, a survival benefit can be achieved in patients who are re-irradiated at first progression, however, this cannot feasibly be offered to some patients (Janssens et al., 2017). Many trials have explored the role of chemotherapy; however, none have been shown to significantly improve survival (Hargrave et al., 2006).

The location of the tumour is the most important determinant of its severe neurological burden. The pons, which means *‘bridge”* in Latin, resides between the cerebellum, the cerebrum and spinal cord as the central component of the brainstem. It contains crossing cerebellar fibres, descending corticospinal fibres, ascending sensory pathways, trigeminal, abducens, facial, vestibular and acoustic nuclei (Figure 1.2). The dorsal pons forms the floor of the fourth ventricle. Consequently, disease arising within the pons can cause profound neurological disability and

obstructive hydrocephalus due to invasion to these vital intrinsic structures or blockage and CSF flow.

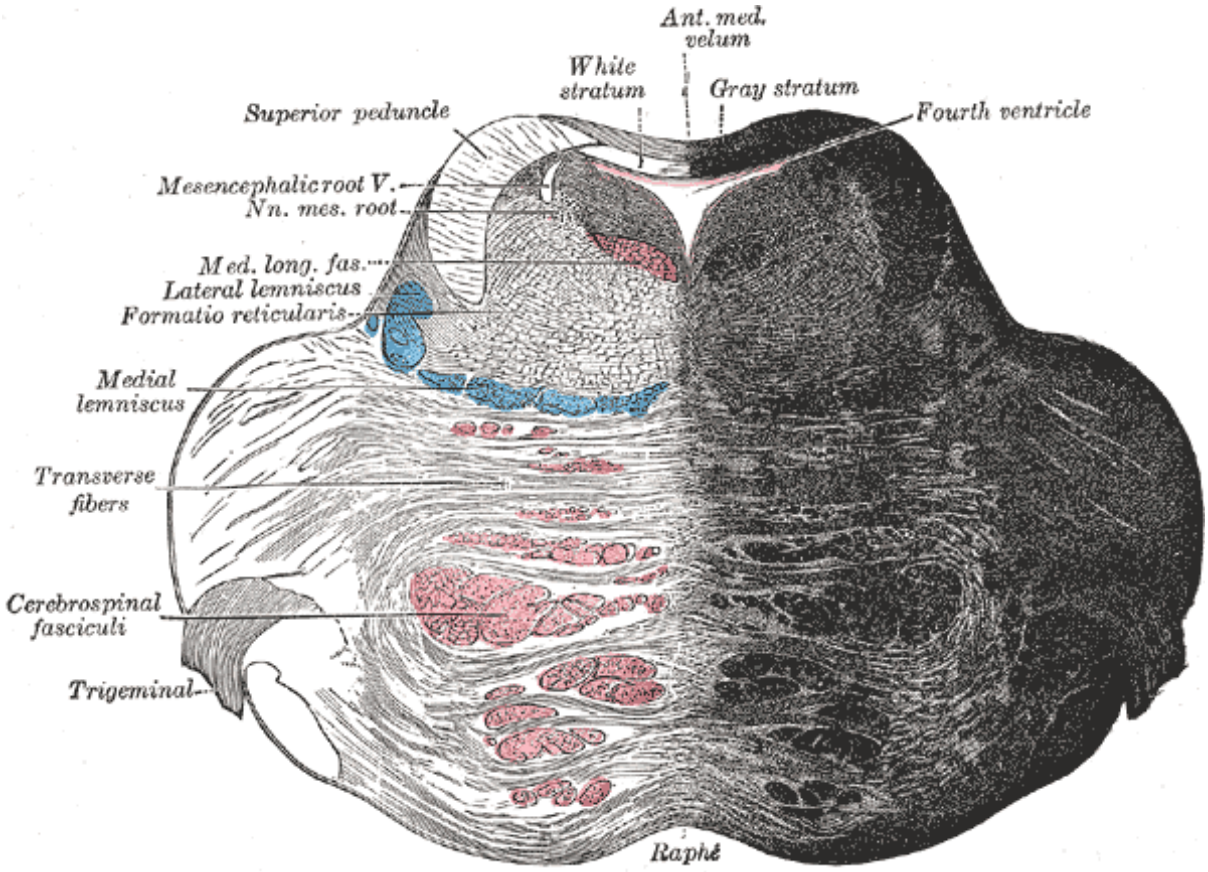

Figure 1.2. A cross-section of the pons at the level of the trigeminal nerve nucleus. Adapted from Gray's Anatomy (1958)

Disease progression is associated with severe neurological disability. The most common symptoms in end stage disease include impaired mobility, dysphagia and dysarthria, with some children developing locked in syndrome with total paresis and the inability to speak or swallow (Veldhuijzen van Zanten et al. 2015; Masuzawa et al., 1993). Consequently, the young age at diagnosis, the lack of treatment options, the rapid progression toward severe disability and death render DIPG a particularly cruel disease.

However, despite the bleak outlook, DIPG is poised for progress (Warren, 2012). For

a long time, progress in DIPG research has been stunted by a lack of available tissue for research (Louis et al., 1993; Cheng et al., 1999; Gilbertson et al., 2003). The neurological morbidity of brain stem biopsy and development of magnetic resonance imaging meant that for a time DIPG became a radiological diagnosis. However, with increasing capabilities to perform genomic analysis on biopsy samples and identify druggable targets, there is an appetite for the return of brainstem biopsy (Kieran et al 2015; Rutka 2012; Macdonald et al., 2012). Through collaborative biological and genomic studies, it is now understood that DIPGs are distinct from adult high-grade gliomas and supratentorial paediatric high-grade gliomas (Mackay et al., 2017). DIPG possesses significant intra-tumoural heterogeneity consisting of different tumour subpopulations that contribute to tumour-genicity and treatment resistance (Vinci et al., 2018). Indeed, it has been demonstrated that DIPG is characterised by K27M mutations in H3 genes, H3FA and the *HIST1H3B* (Wu et al., 2012; Quong-Quang et al 2012; Schwartzentruber et al 2012). This has led to the reclassification of DIPG as Midline Diffuse Glioma H3K27M by the World Health Organisation (Louis et al., 2016). This reclassification groups DIPG together with midline tumours bearing the same type of mutation located outside the pons, often in the thalamus. It is hoped that drugs that will be designed to functionally target these mutations and will lead to better outcomes for patients. Indeed, genetic classification may lead to different treatment strategies appropriate to their risk profile. Medulloblastoma has been reclassified based on molecular profiles (Northcott et al, 2011), with low risk subtypes being subjected to trials comparing reduced intensity treatment strategies versus standard therapy (NCT02724579; ANCNS1422). Nevertheless, the association between genetic subtypes and prognosis strongly suggest that H3K27 mutations are closely related to

clinically relevant disease behaviour. But despite, the therapeutic potential of molecular phenotyping, DIPG is still a useful term. Even amongst H3K27M Midline Diffuse Gliomas, the outcomes of brainstem tumours are still poorer than their supra-tentorial counterparts (Mackay et al., 2017), suggesting that there are factors beyond genetics that are still important. Indeed, it is likely that that much of this lethality of DIPG compared to many supratentorial high grade gliomas is their anatomical location.

Chronic intermittent convection enhanced delivery in DIPG and the Functional Neurosurgery Group

Despite the hopes surrounding targeted therapy, penetrating the BBB still remains a hurdle for any new drug intended to treat DIPG and wider CNS disease. CED has become an important avenue for exploration for the future of DIPG. Several trials are underway (NCT03086616; NCT03566199; NCT01502917). The only trial published to date using CED in DIPG is a landmark phase I dose escalation study delivering an ^{124}I isotope conjugated to an antibody targeting B7-H3 antigen found on DIPG cells (Souweidane et al., 2018). The study reported findings from 28 patients and demonstrated the principle of using CED to achieve high intra-lesional dosing with negligible systemic exposure. In this trial between 0.24 and 4.4 mL were infused over 1.18 -16 hours. Of the 25 who reached the primary end point of the study, 25 patients had a median overall survival of 15.3 months. The treatment was tolerated; however, there were 275 all cause adverse events, which emphasizes the need to better understand how morbidity can be reduced. Most events were mild to moderate (251/271). Using Common Terminology in Criteria for Adverse Events (CTCAE Version 5.0) the most common adverse events were hyperglycaemia, reduced

lymphocytes and decreased white blood cells reported in 27, 24 and 19 instances respectively. There was one instance of life threatening respiratory failure. A limitation of the CTCAE in describing toxicity arising from brain stem CED is that is has been designed to evaluate systemic therapy and does not emphasize the importance of anatomical targeting, which is central to CED.

When adverse events are grouped together by system adverse events were commonly neurological, which were reported in 69 instances - accounting for 25% of total adverse events recorded (Figure 1.3). Furthermore, when it is considered that the brain stem controls cardiovascular and respiratory functions, as well as housing various essential neural structures, it is evident that even more adverse events could be attributed to brain stem dysfunction as a whole (Figure 1.3). Indeed, some recorded side-effects could also be due to combinations of neurological deficits such as dysarthria arising from bulbar dysfunction or cerebellar dysfunction. This is less important when evaluating systemic therapy because delivery to the brain will be assumed to be uniform. When we consider the interaction between brain-architecture, drug concentrations, local tissue pressures and surgical injury these distinctions become important to understand and prevent toxicity. Moreover, a significant part of the complex neurology recorded could be due to disease progression, the extent to which the CTCAE can differentiate between treatment-related neurological deterioration and disease-related deterioration is limited. Development of the technique requires a better understanding these problems and how their impact can be mitigated, which may involve a revision of how we describe toxicity for CED altogether.

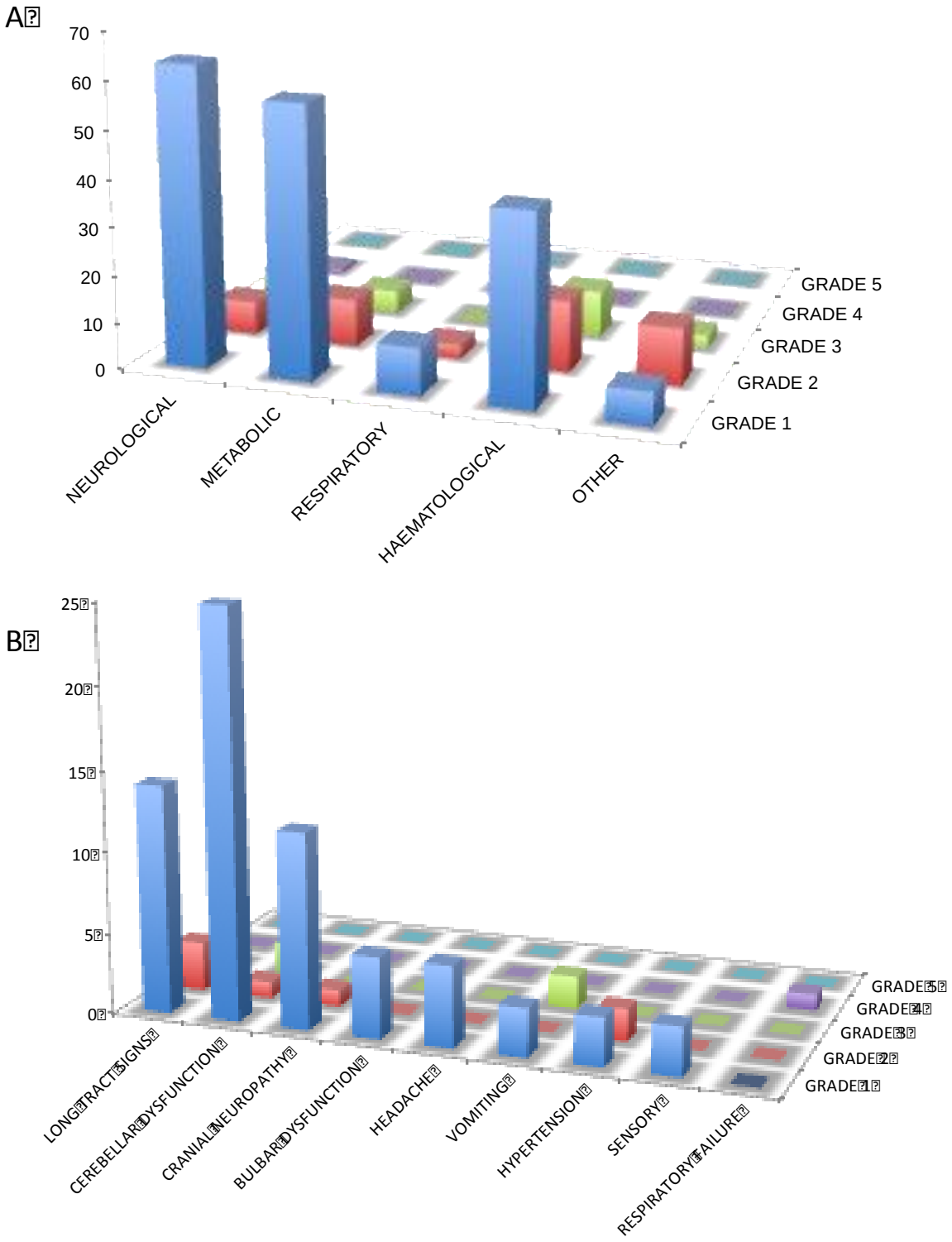

Figure 1.3. Grouping of adverse events reported in the Convection-enhanced delivery for diffuse intrinsic pontine glioma: a single-centre, dose-escalation, Phase 1 trial (Souweidane et al., 2018). Adverse events are grouped based on system affected, demonstrating that neurological side-effects account for a significant proportion of morbidity (A). Adverse events attributable to brain stem function are further described, demonstrating the complex neurological phenotype of patients with DIPG receiving CED (B)

Another consideration regarding work by Souweidane et al., 2018 is that this method of delivery can only be used on a limited number occasion, usually only once. This technique suits the infused agent, which uses a antibody-conjugated isotope, which has a long half-life and prolonged radio-sensitizing effect. When using a pharmacological agent, such as the many proposed targeted therapies for DIPG, it would advantageous to perform intermittent delivery so high intra-parenchymal concentrations of drug can be maintained for longer. The first attempts to treat DIPG with intermittent CED by the Functional Neurosurgery Group at the University of Bristol involved stereotactically implanting a guide tube into the pons, which allowed repeated access the pons via the same trajectory (Barua et al., 2013). Prior to each infusion, the drug delivery catheter would be inserted through the guide tube to target. The distal catheter would be tunnelled out through the skin and connected to a drug administration pump allowing pontine infusion in the awake patient. When infusion was completed the drug delivery catheter would be removed during a short operation. When the patient had recovered from pontine infusion the pons could then be re-accessed via the indwelling guide tube. This method enabled repeated delivery of 8.7-14.2 mL of carboplatin directly into the tumour over 20-24 hours on 5 separate occasions.

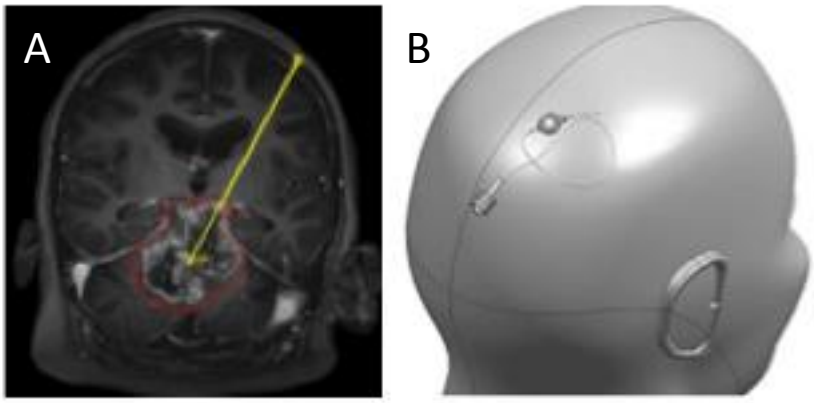

Figure 1.4. Intermittent convection enhanced delivery in a patient with diffuse intrinsic pontine glioma using an indwelling guide tube. A recessed-stepped catheter system would be inserted stereotactically into the tumour leaving behind the outer guide tube (A). At infusion, a catheter would be inserted to target and the distal catheter would be tunnelled through the scalp and connected to an external infusion pump (B). Adapted from Barua et al. 2013

However, this approach was limited by the need for two operations for each pontine infusion and the repeated catheterisation of the brain stem. This would increase the risk of surgical complications and reactive gliosis around the catheter track, which could impair drug distribution. To overcome these limitations a chronic intermittent CED system using a transcutaneous bone-anchored port (TBP) was developed in large animals before being translated into humans with GBM and Parkinson's Disease (Bienneman et al., 2012; Barua et al., 2016; Whone et a., 2019). This drug delivery system was adapted for use in DIPG using a system of 4 implantable micro-catheters, targeting the pons via trans-frontal and trans-cerebellar trajectories connected to the TBAP by sub-galeal tubing. Initially, 8 patients (ages 4–12 years) with DIPG were infused with up to 9 cycles of carboplatin divided over two consecutive days at a concentration of 0.18 mg/ml (Singleton et al. 2016). These infusions were associated with neurological side effects, which were most commonly reported during the first cycles. This thesis describes the ongoing development of

this technique using sodium valproate, as a targeted therapy for DIPG (Killick-Cole et al., 2017), as a single therapy and in combination with carboplatin in 15 children treated on compassionate grounds.

Table 1. 1. All cause adverse events from Convection-enhanced delivery for diffuse intrinsic pontine glioma: a single-centre, dose-escalation, Phase 1 trial using the Common Terminology Criteria for Adverse Events Version 5.0 (Souweidane et al., 2018).

Adverse Event	Grade 1	Grade 2	Grade 3	Grade 4
Abducens palsy*	3 (11%)	0	0	0
Agitation*	2 (7%)	2 (7%)	0	0
Alanine aminotransferase increase	9 (32%)	0	0	0
Anaemia	3 (11%)	6 (21%)	0	0
Ankle clonus*	3 (11%)	0	0	0
Anxiety	2 (7%)	2 (7%)	0	0
Aspartate aminotransferase increase	5 (18%)	2 (7%)	0	0
Ataxia*	7 (25%)	0	1 (4%)	0
Cough	3 (11%)	0	0	0
Decreased rapid alternating movements*	3 (11%)	0	0	0
Diplopia*	6 (21%)	1 (4%)	0	0
Dysarthria*	3 (11%)	0	1 (4%)	0
Dysmetria*	5 (18%)	0	0	0
Dysphagia*	2 (7%)	1 (4%)	0	0
Facial palsy*	9 (32%)	1 (4%)	0	0
Fatigue	3 (11%)	0	0	0
Gait disturbance*	4 (14%)	1 (4%)	0	0
Headache*	8 (29%)	6 (21%)	0	0
Haemoglobin increased	5 (18%)	0	0	0
Hyperglycaemia	17 (61%)	10 (36%)	0	0
Hypernatraemia	6 (21%)	0	0	0
Hypertension	3 (11%)	2 (7%)	0	0
Hypoalbuminaemia	17 (61%)	0	0	0
Hypocalcaemia	4 (14%)	0	2 (7%)	0
Hypokalaemia	4 (14%)	0	3 (11%)	0
International normalised ratio increased	10 (36%)	0	0	0
Lymphocyte count decreased	5 (18%)	9 (32%)	10 (36%)	0
Muscle weakness, left-sided*	4 (14%)	1 (4%)	1 (4%)	0
Muscle weakness, right-sided*	3 (11%)	1 (4%)	2 (7%)	0
Nasal congestion	5 (18%)	1 (4%)	0	0
Neutrophil count decreased	7 (25%)	8 (29%)	0	0
Nystagmus*	3 (11%)	0	0	0
Pain	6 (21%)	3 (11%)	0	0
Paraesthesia	3 (11%)	0	0	0
Platelet count decreased	7 (25%)	0	0	0
Rash, maculo-papular	2 (7%)	1 (4%)	0	0
Respiratory failure	0	0	0	1 (4%)
Skin infection	1 (4%)	0	1 (4%) 0	0
Vomiting	3 (11%)	2 (7%)	0	0
White blood cells decreased	13 (46%)	6 (21%)	0	0

* neurological adverse events

Chapter 2. The treatment of patients with sodium valproate administered by chronic intermittent convection enhanced delivery for diffuse intrinsic pontine glioma

Background

Around 80% of diffuse intrinsic pontine glioma (DIPG) possess mutations in genes encoding histone proteins (Wu et al. 2012, Schwartzentruber et al. 2012, Khuong-Quang et al. 2012, Sturm et al. 2012), Histones are proteins that form nucleosomes packaging deoxy-ribonucleic acids (DNA) into chromatin. Histones belong to five major subgroups, H1/5, H2A, H5 and H3, the latter of which is encoded by genes HIST1H3B or H3F3A and are mutated in DIPG (Wu et al. 2012, Schwartzentruber et al. 2012, Khuong-Quang et al. 2012, Sturm et al. 2012). Histone proteins bind together to form octameric complexes and undergo epigenetic modification to control gene expression (Lorch, LaPointe and Kornberg 1987). Epigenetic modification of histones is dependent on the enzymatic addition and removal of chemical groups - a process known as covalent modification. This becomes heavily dysregulated in DIPG (Lewis et al. 2013, Bender et al. 2013). Therefore, a possible strategy to improve outcomes for patients with DIPG is to functionally target disordered epigenetic regulation (Grasso et al. 2015). An important group of enzymes involved in covalent modification of histones are histone deacetylases (HDAC), which remove acetyl groups from histone lysine residues. As reviewed by Glozak and Seto, 2007, this process can neutralize positive charge to relax chromatin structure and increase bromodomains to activate gene transcription. HDACs have numerous roles in oncogenesis including inhibition of transcription in favour of proliferation, repression of pro-apoptotic factors and inhibition of cell cycle check-point molecules. Grasso et al., 2015 demonstrated that Panobinostat, as an FDA-approved pan-HDAC inhibitor

(HDACi), was a potent agent against DIPG cells in vitro and in rodent xenografts. Panobinostat is now subject to an ongoing clinical trial (NCT02717455).

However, other inexpensive drugs currently in clinical use also have HDACi properties including anti-epilepsy drug, sodium valproate. The HDACi characteristics of sodium valproate give rise to its teratogenic properties in pregnancy and also its anti-cancer activity (Phiel et al. 2001, Brodie and Brandes 2014). Patients with DIPG treated for seizures with sodium valproate have been shown to survive longer (Felix et al. 2011, Felix et al. 2014, Masoudi et al. 2008). However, like many drugs, sodium valproate penetration into the central nervous system is limited and reduces intra-tumoural concentration. It is estimated that only between 6% and 20% of the sodium valproate serum concentration is found in the brain parenchyma (Vajda et al. 1981, Kim et al. 2013, Wieser, 1991). Increasing systemic administration may overcome such a problem; however, at high doses sodium valproate causes drowsiness, mood disturbance, thrombocytopenia, bone marrow suppression, weight gain and hair loss (Sztajnkrycer, 2002). In order to achieve high intra-tumoural concentration and reduce systemic toxicity, local delivery of sodium valproate using CED may be advantageous. Sodium valproate is water soluble and readily compatible with CED. We hypothesised that sodium valproate could be delivered by chronic intermittent CED to provide local control of disease. We tested this hypothesis in a convenience pilot cohort of children with DIPG treated on compassionate grounds.

Methods

Patients were referred internationally from paediatric oncology centres. Patients were offered treatment with sodium valproate CED as a single agent on compassionate grounds if they were considered eligible. Eligibility was determined on the basis of the patient's clinical status, radiological signs consistent with DIPG and a total disease burden confined to the brainstem within a volume coverable by two trans-frontal and two trans-cerebellar catheters. Patients of poor clinical status, metastatic disease or with cysts and haemorrhage obstructing catheter implantation were not offered treatment.

Ethics and consent

Implantation of the drug delivery system was approved by the Medical and Healthcare products Regulatory Agency. Treatment with chronic intermittent CED, including implantation of the drug delivery system and brain stem infusion, was subject to local independent ethical approval at the University Hospital Bristol NHS Trust and Harley Street Clinic. Parents were consented for the experimental nature of the treatment including potentially unpredictable and severe complications.

Drug delivery system implantation

Patients underwent magnetic resonance imaging (MRI) under general anaesthetic to allow planning of catheter trajectories and the transcutaneous bone anchored port (TABP). Drug delivery system implantation was planned using an in-house module of neuro|inspire® neurosurgical planning software (Renishaw, Wooton-under-Edge) as follows:

- the tumour boundary was traced using fluid attenuated inversion recovery (FLAIR) imaging
- Bilateral trans-frontal and trans-cerebellar trajectories were planned to target the ventrolateral pons and the central/dorsal pons respectively avoiding blood vessels
- Step-length from the catheter outer guide tube was increased to 35mm or the grey-white matter interface, whichever smallest
- Recess step between the inner and outer guide tube was 10 mm.
- The TBAP was planned in the parietal bone superior to the asterion

The method of implanting the drug delivery system has been previously described (Barua et al. 2016). In short, patients would be placed under general anaesthetic and their head fixed in Leksell® stereotactic frame. The patient would undergo pre-operative computer tomography (CT) angiogram, which would then be co-registered to the pre-operative MRI using neuro|inspire® software. The patient would be placed prone with the Leksell frame fixed to the neuro|mate® stereotactic robot (Renishaw Plc; Figure 2.1). After necessary sterile precautions and administration of antibiotics, stereotactic co-ordinates would then be exported to the stereotactic robot to execute the first trajectory. Here, a scalp incision would be made and the periosteum retracted, a multi-featured burr hole would be drilled. Using a specialised suite of instruments, a 1 mm guide rod would be passed along the trajectory to breach the grey/white interface. A guide rod with an outer diameter of 0.6 mm would then be passed to target. A recessed-step catheter system (Figure 2.1) would then press fit into the multi-featured burr hole followed by a stylet to keep the catheter tract patent. This would be repeated for each trajectory followed by implantation of the TBAP. The

TBAP would be sited through a hole punched in the skin, subcutaneous tissue would be excised using a surgical aspirator preserving the underlying periosteum. Around the hole a semi-circular flap would be raised with the base of the flap sparing the occipital arteries. The skull would then be drilled to accommodate the TBAP. After this, the TBAP, would be connected to a tubing manifold, which would then be connected to subgaleal tubing. Subgaleal tubing would be tunnelled to the respective catheters and primed with artificial cerebral spinal fluid (aCSF). Four carbothane catheters would be cut to length and each connected to a subgaleal tube. These would be inserted to target whilst infusing aCSF. Once in place, the subgaleal tubing would be shortened and reconnected to the manifold. Wounds would be closed in layers.

Pontine infusion

Pontine infusion was initiated within 72 hours of implantation of the drug delivery system. Prior to infusion patients would undergo pre-infusion MRI to exclude contra-indications such as brainstem haemorrhage or hydrocephalus. Patients were examined by the attending neuro-oncologist. Consent for infusion would be gained from the parent or guardian. Pontine infusion would be conducted in the Paediatric Intensive Care Unit (PICU). A needle administration set would be connected to extension lines, which were connected to an external B-Braun pump (Figure 2.1). The lines would be primed with drug and attached to an actuator base. The base would be applied to the TBAP, all catheters would be infused starting with a ramping regime as follows:

- 0.03ml/min/catheter for 10 min,
- 0.06 ml/hour/catheter for 5 min,
- 0.12 ml/min/catheter for 5 min,
- 0.18 ml/min/catheter for 5 min,
- 0.18-0.3 ml/min/catheter until completion

Infusion would be stopped at the onset of significant neurological deficit according to the attending paediatric neuro-oncologist/neurosurgeon. 10 patients were treated (Patients A-J). Sodium valproate was delivered in Patient A at 14.4mg/ml before being escalated to 28.8 mg/ml after the first infusion. Infusions were conducted at 28.8mg/ml thereafter. Patient D received sodium valproate at 21.6mg/ml due to concerns about concentration related toxicity.

Pontine Infusions were conducted over two consecutive days as part of a single treatment cycle. Treatment cycles were repeated at 4-8 week intervals depending on patient fitness. Post-infusion MRI was performed at the end of the first day's infusion.

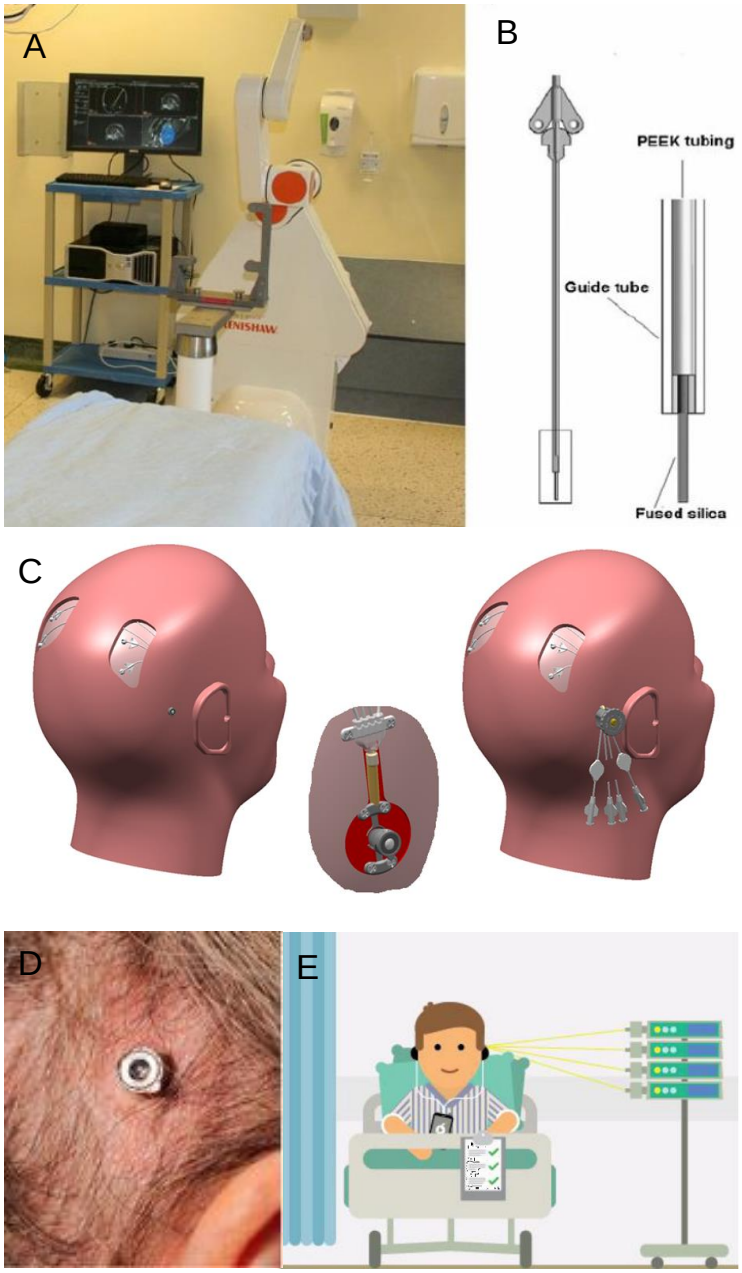

Figure 2.1. A robot-guided chronic, intermittent drug delivery with a transcutaneous bone anchored port (TBAP) for awake pontine infusion. A. neuro|inspire® neurosurgical planning software (Renishaw, PLC, Wooton under Edge), is used alongside the Neuro|mate stereotactic robot (Renishaw PLC) to site the drug delivery system according to pre-operative imaging (Barua et al. 2013); B. A recessed stepped catheter system using an inner and outer guide tube is inserted into the brain to facilitate controlled reflux and aid homogenous drug distribution (Barua et al. 2013); C. Schematic diagrams of the drug delivery system in situ, consisting of skull anchored catheters, subgaleal tubing connected to a manifold and TBAP, which allows the connection of an administration set; D. The TBAP in situ in an adult patient (Barua et al. 2016a); E. Child-friendly diagram of awake pontine infusion via the chronic, implantable drug delivery system, courtesy of Harley Street Clinic, Health Corporation, America

Results

10 patients with DIPG were accepted for drug delivery system implantation and treatment with sodium valproate monotherapy delivered by CED (Table 2.1). 3 patients had DIPG with confirmed H3K27M DIPG. All patients, except Patient E received radiotherapy prior to implantation. Patient C received proton beam therapy and Patient D had hypo-fractionated radiotherapy. In addition to CED, patients also received a variety of additional experimental treatments (Table 2.1). Patients were implanted at a median of 4.6 months (range 3.3-10.1 months) following diagnosis.

Surgical implantation of the drug delivery system was well tolerated by all patients except Patient E. Patient E was implanted prior to radiotherapy. This patient had H3K27M positive midline diffuse glioma and was implanted 3.6 months following diagnosis. The implantation was complicated by intra-operative extubation and hypertension. Post-operatively, the patient had generalized brain oedema with micro-haemorrhage away from the catheter trajectory resulting in neurological deterioration requiring hypertonic saline. Her clinical status contraindicated further pontine infusion. The patient improved over the intervening 2 weeks and she began radiotherapy 2 weeks later. After 3 fractions of radiotherapy at 1.8 Gy, the patient was re-anaesthetised for an unrelated urological procedure but sustained another period of intra-operative hypertension and suffered brain stem haemorrhage. Patient E died 4.1 months from diagnosis.

Pontine infusions were associated with neurological deterioration. Patient A developed left facial weakness during her first infusion, which recovered immediately upon stopping the infusion suggesting it was directly infusion-related. Review of catheter position demonstrated that the right frontal catheter terminated deep to the

left facial colliculus. This catheter was retracted and further infusions were associated with less severe facial weakness. Each subsequent infusion was associated with mild-moderate cerebellar symptoms, mild right-sided hemiparesis and left 6th nerve palsy, all of which recovered between cycles. Unlike the initial facial palsy, these deficits could not be ascribed to a specific catheter. Subsequent patients who received sodium valproate by CED also developed neurological symptoms during infusion, which included hemiparesis, cerebellar ataxia, trigeminal dysesthesia, facial nerve and abducens nerve palsies. Most neurological deficits recovered. Patent B suddenly deteriorated at the beginning of infusion requiring cessation of infusion and immediate CT imaging. There was no evidence of acute intracranial event such as hydrocephalus or brain stem haemorrhage. The patient went onto make a slow recovery but remained wheelchair bound thereafter. Patient D suffered left sided hemiparesis repeatedly during infusions and after the second infusion failed to recover to pre-infusion baseline. Patient D went onto receive a lower concentration of sodium valproate at 21.6mg/mL; however, this was associated with a similar side-effect profile. Patient F also developed ataxia during infusion, which also failed to return to baseline following infusion.

Median overall survival from diagnosis was 14.4 months (range 4.1 - 23.6). Response following treatment with sodium valproate by CED was observed in Patient A after 2 cycles of CED (Figure 2.2). However, at 13.3 months following diagnosis, MRI demonstrated evidence of progressive disease. The patient was referred for palliative radiotherapy followed by a final cycle of CED at 18 months. At 19 months, the patient rapidly deteriorated and died, 20 months after diagnosis. Patients C, F G and H developed progression of disease within 2 months of receiving CED. Due to the observed lack of efficacy in these cases, patients still

receiving treatment were switched onto sodium valproate therapy combined with carboplatin (Chapter 4).

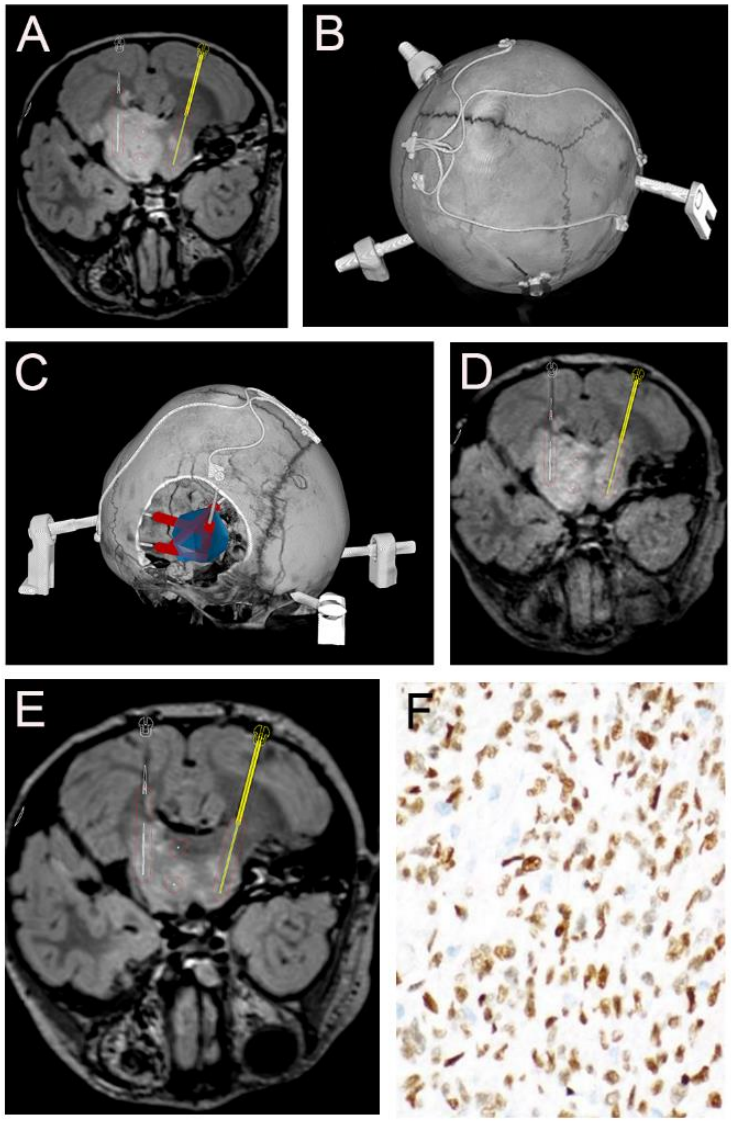

Figure 2.2. Chronic intermittent convection enhanced delivery of sodium valproate for the first in human treatment of diffuse intrinsic pontine glioma (Patient A). Pre-implantation axial Fluid Attenuated Inversion Recovery (FLAIR) sequences along the planned trajectory of the left cerebellar catheter (yellow) demonstrated pre-implantation (A). Post-operative imaging demonstrates implantation of a chronic implantable drug delivery system connecting a bone-anchored drug administration port to 4 catheters by sub-galeal tubing (B). The trans-frontal and trans-cerebellar catheters target the tumour (blue) delivering drug along their distal trajectory (red) using a recessed-step catheter design (C). Intra-parenchymal infusion of sodium valproate was performed demonstrating hyperintensity on FLAIR sequences around each catheter (D). After two cycles of treatment, disease response was demonstrated by reduction in pontine volume and FLAIR hyperintensity (E). Post mortem histopathological analysis demonstrated positivity for H3 K27M at x20 magnification using immune-histochemical staining (F).

Discussion

10 patients with DIPG underwent implantation of the drug delivery system, 9 of whom went onto receive pontine infusion of sodium valproate. Median overall survival for this cohort was longer than expected compared to the wider DIPG population. Surgical implantation was associated with one severe adverse event, but was otherwise well tolerated. Pontine infusions were associated with neurological deterioration, which in the most part was transitory; however, three patients sustained moderate to severe neurological deficits that failed to recover completely. How the mortality and morbidity of this treatment could be managed to improve survival and quality of life in patients is discussed.

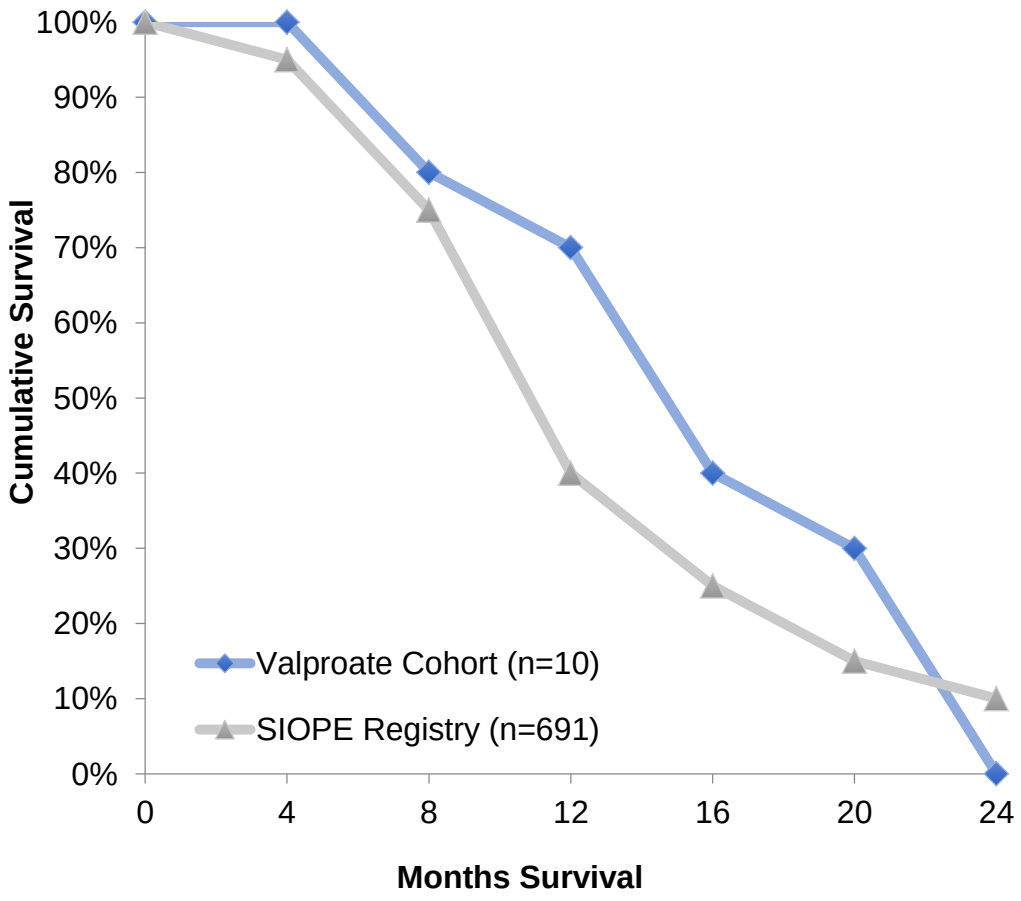

Figure 2.3. Survival curves for 10 children with diffuse intrinsic pontine glioma (DIPG) treated with sodium valproate administered by chronic intermittent convection enhanced delivery versus the SIOPE DIPG Registry (Veldhuijzen van Zanten et al., 2017)

Multiple infusions were performed without life-threatening side-effects and most patients tolerated infusions and surgical implantation well. One response following treatment was identified demonstrating encouraging signs of efficacy. However, the extent to which we can attribute this, or the longer than expected survival in these patients, to CED is limited. Patient A received veliparib as part of a clinical trial (NCT01514201) and left the trial after being diagnosed with tumour progression. In retrospect, this may have represented pseudo-progression, which is clinically and radiologically indistinguishable from disease progression. Pseudo-progression represents an inflammatory response following tumour treatment and is associated with a more favourable prognosis (Carceller et al. 2016). Reduction in tumour volume after initiation of CED may have represented remission of this inflammatory process rather than response to treatment. Indeed, considering that patients had to be clinically fit to undergo surgery and selection of patients was subject to various clinical, anatomical and pathological considerations, there is a significant risk of selection bias. As such, the favourable response in one patient and the better than expected survival overall should be interpreted with caution and further treatment as part of a clinical trial should be prioritized. Most importantly, this preliminary experience demonstrates that chronic intermittent CED of sodium valproate can be feasibly performed in patients with DIPG. Moreover, it identifies the important challenges regarding safety of ongoing treatment.

Most notably there was one death during treatment not attributable to progression of disease and thus must represent a central point for discussion in how the safety of pontine CED can be improved. Patient E's ultimate deterioration occurred 3 weeks following initial implantation during an unrelated urological procedure under general anaesthetic performed whilst undergoing radiotherapy. It must be acknowledged that

children with brain tumours who have received radiotherapy have increased baseline risk of stroke, this is 100-fold but often as a delayed effect (Campen et al. 2012, Greene-Schloesser et al. 2012). Equally, patients with DIPG are at high risk of death- approximately 5% of patients with H3FA DIPG are dead by 4 months (Veldhuijzen van Zanten et al. 2017). However, the extent to which implantation could have contributed to this severe adverse event must be explored. Surgical intervention in these patients is known to be high risk. Historically, surgical resection has been avoided in DIPG due to excessive morbidity and lack of efficacy (Epstein and McCleary 1986). Stereotactic brain stem biopsy in a contemporary cohort of 130 patients with DIPG is associated with a morbidity of 3.4% (Puget et al. 2015). Therefore, it can be expected that experimental surgery of this nature will be associated with significant risk. However, the tolerance of Patient E to surgery is dramatically contrasted with the good post-operative outcomes of other patients implanted using the same drug delivery system. As such, it is essential to reflect on how risk can be reduced in the future.

Patient E's surgery was remarkable for hypertension intra-operatively. It is understood that hypertension is a risk factor for intracerebral haemorrhage after cranial neurosurgery (Basali et al. 2000). It is possible therefore that intra-operative hypertension contributed to the eventual brain stem haemorrhage. Autonomic control is regulated by the central autonomic network, which consists of interconnected neural systems in the telencephalon, diencephalon and brainstem that co-ordinate sympathetic and parasympathetic output (Bennaroch 2012). The brain stem contains the nucleus of the solitary tract, the nucleus ambiguus and the dorsal motor nucleus of the vagus nerve, all of which form fundamental parts of the central autonomic network (Bennaroch 2012). Experimental studies in animals demonstrate that

bilateral lesioning of the nucleus of the solitary tract produces acute hypertension, blood pressure lability, chronic hypertension and exaggerated hypertensive responses to environmental stress (Doba and Reis 1973, Nathan and Reis 1977). It is feasible therefore that physiological challenges arising from surgery and anaesthetic combined with instrumentation of brainstem in DIPG could increase the risk of autonomic dysregulation, hypertension and haemorrhage.

Patient E was also distinct from the other patients by the absence of pre-implantation radiotherapy. It is possible that radiotherapy could act to reduce the risk of bleeding during drug delivery system implantation. Blood supply to the pons is provided by perforating arteries of the basilar artery and its branches. Endothelial cells are exquisitely sensitive to radiation ultimately resulting in reduction of the vascular network and ischaemia (Venkatasulu et al. 2018). Outside the brain, the acute effects of radiation can be used as a palliative procedure to control persistent tumoural bleeding (Kondoh et al., 2015). As such, it is possible that the contrasting surgical outcomes between Patient E and the rest of the cohort could be attributed to this effect of radiotherapy. Without further evidence conducted within a clinical trial, this is speculative. Nevertheless, at this early critical stage of developing CED for DIPG, further treatment of patients should be performed after radiotherapy.

Lessons from Patient E demonstrate clear points about how to reduce surgical morbidity; however, the infusions were also associated with toxicity. Considering that patients receive multiple infusions during treatment, these represent important sources of morbidity for the child. All patients experienced some degree of neurological disability during infusion, which reportedly resolved within hours to days. Previously, neurological side-effects have been reported to occur after pontine

infusion as a delayed effect (Singleton et al. 2016) but this was not observed in this cohort. Significant side-effects were observed in Patient B, D and F who both developed disabling neurological side-effects during infusion that failed to recover to pre-infusion baseline. The cause of such side-effects could relate to the toxicity of the drug, the physiological effect of infusion or their interaction with the underlying tumour. Nevertheless, the impact of permanent neurological side-effects in DIPG, where quality of life is paramount, should be minimised. It is positive that such events only occurred in the minority of patients. However, the side-effects experienced by other patients at the time of infusion were indistinguishable from those that failed to recover in Patient D and F. As such, our ability to predict recovery from infusion-related deficits is limited and as a consequence the attending physician has to weigh the unknown risk of permanent deficit associated with continuing infusion versus the theoretical benefit of maximising infusion volume to increase the volume of treated disease. Balancing unknown risks versus unknown benefits is common when developing new treatment. However, particular to awake pontine infusion is that the calculation of risk versus benefit is made continually as side-effects evolve in real time during drug administration. Managing such uncertainty with such frequency, therefore, presents many challenges for the child, the parent and the team treating the patient. Improving our understanding of the clinical manifestations of pontine infusion therefore represent a priority for the development of pontine CED for DIPG.

Table 2.1. Characteristics of 10 patients accepted for drug delivery system implantation for treatment with valproate monotherapy

Patient	Age at diagnosis (years)	Biopsy	Diagnosis to implant (months)	CED cycles (infusion) valproate	Additional CED therapies	Additional treatment	Survival from diagnosis (months)
A	4	H3 K27M	6.1	5 (9)	-	54 Gy Rtx, Veliparib	20
B	7.7	-	10.1	1 (1)	Panobinostat	Newcastle disease virus, gallium maltholate, oral panobinostat, avastin	23.6
C	5.6	-	4.3	4(2)	Separate Valproate Carboplatin	Proton Beam therapy, oral metformin	14.9
D	10.6	-	4.9	5 (10)	Combined Valproate Carboplatin	34 Gy RTx, immunotherapy, palliative radiotherapy	17.8
E	7.3	H3 K27M	3.6	0	-	5.4 Gy Rtx (post-implant)	4.1
F	11.2	-	5	2(3)	-	54 Gy Rtx, Immunotherapy, intra-arterial therapy	23.5
G	8	H3 K27M	3.5	2(3)	Carboplatin	54 Gy Rtx, intra-arterial therapy	9.0
H	9.3	H3 K27M	4.3	1	Separate Valproate and Carboplatin	54 Gy Rtx	6.4
I	7.8	-	7.9	1 (1)	Combined Valproate Carboplatin	54 Gy Rtx , 4 cycles carboplatin	13.9
J	6.9	-	3.3	4 (8)	Combined Valproate Carboplatin	54 Gy Rtx, palliative radiotherapy	13.5

Chapter 3 Development of a neurological scale to monitor patients during pontine infusion

Background

Treating patients with diffuse intrinsic pontine glioma (DIPG) on compassionate ground using awake pontine infusion represents an important opportunity for the development as CED as a treatment for neurological disease. Repeated infusions of chemotherapy directly into the brainstem were feasible and many patients achieved favourable outcomes. On the other hand, this new treatment was accompanied by new patterns of treatment-related toxicity, which need to be better understood. Patients developed neurological deficits at the time of administration and their recovery was unpredictable. This neurological toxicity places additional burden on the patient, when quality of life is paramount. It complicates the design of treatment schedules requiring intensive monitoring in a paediatric intensive care unit (PICU), which increases costs and reduces availability. Furthermore, the attending physician has to balance risks and benefits in real-time weighing the risk of permanent deficit against the desire to control disease. Quantifying and controlling neurological toxicity occurring during pontine CED would therefore represent a major advance in the development of the treatment.

Complex decision making about risks and benefits is nothing new in translational medicine. Karnofsky et al, performed a study on patients with similarly inoperable, untreatable cancer in 1948 using a new chemotherapy, nitrogen mustard (Karnofsky et al. 1948). It was difficult to quantify the clinical benefit of treatment because despite there being '*subjective and objective evidence of improvement'* many patients remained *'bedridden'* (Karnofsky and Burchenal, 1949). Consequently, the

burden of the treatment remained difficult to justify and the types of patients most likely to benefit remained unknown. This led to the concept of Karnofsky's Performance Status (KPS), which measures functional impairment between 0-100 with lower scores indicating worse function. This enables clinical decisions to be made regarding fitness for treatment, determine prognosis and compare patients between studies. Although Karnofsky's Performance status represented an advance in how to translate new treatments, the way toxicity of new treatments were described was also problematic.

Prior to 1982, toxicity during cancer treatment was described using ad hoc and inconsistent descriptions. The Acute Radiation Morbidity Scoring Criteria and Common Toxicity Criteria, which were later amalgamated into the Common Criteria for Adverse Events (CTCAE), provide detailed descriptions of side-effects arising from treatment (Trotti et al. 2000). However, pontine CED causes dynamic neurological changes that recover at varying points post-infusion and may even occur between infusions; this makes methods such as KPS and CTCAE less useful. Souweidane et al., 2018 addressed this problem in their Phase I trial by measuring toxicity at 7 days post infusion. However, such an approach cannot inform the risk-benefit decisions made during infusion because deficits arise in real-time. Continuous neurological assessment during infusion is required to identify neurological deficits at onset and quantify recovery. It is possible to use conventional neurological examination as used in the patients treated with carboplatin and valproate monotherapy. However, infusions were still associated with significant toxicity, and inconsistent technique between assessors and assessments made identifying and communicating onset of deficit and recovery difficult. Patients treated

with CED would therefore benefit from a reliable neurological assessment scale that could be performed before, during and after pontine infusion. This would enable the consistent documentation of neurological change during therapy and inform treatment decisions appropriately. I hypothesized that I could develop a reliable neurological assessment system that could use patient-reported symptoms and observed neurological signs to quantify brain stem dysfunction occurring during pontine infusion.

Methods

A literature search was conducted based on the cardinal signs and symptoms of DIPG: long tract signs, ataxia and cranial neuropathy (Figure 3.1). Only journal articles published within the last 10 years studying humans were included. Articles were excluded if they did not involve clinical assessment scales. Abstracts were reviewed to identify articles that used scales that elicited the cardinal features. Scales were selected if they had been validated in a paediatric population and could be conducted at the bedside without equipment. This left the pediatric National Institutes of Health Stroke Scale (pedNIHSS) (Ichord et al., 2011), the Scale for Assessment and Rating of Ataxia (SARA) (Schmidt-Hübsch et al., 2006), International Co-operative Ataxia Rating Scale (ICARS) (Trouliass et al., 1997) and the Brief Ataxia Rating Scale (Schmamahann et al., 2009). Scales were deconstructed into their individual items (Table 3.1). Items that did not assess long tract signs, cranial neuropathy or ataxia were excluded. Measures of visual function were also excluded due to the location of the optic pathway outside of the pons. Items that could not be completed safely while attached to the infusion set were excluded, i.e. measures of gait, stance and sitting balance.

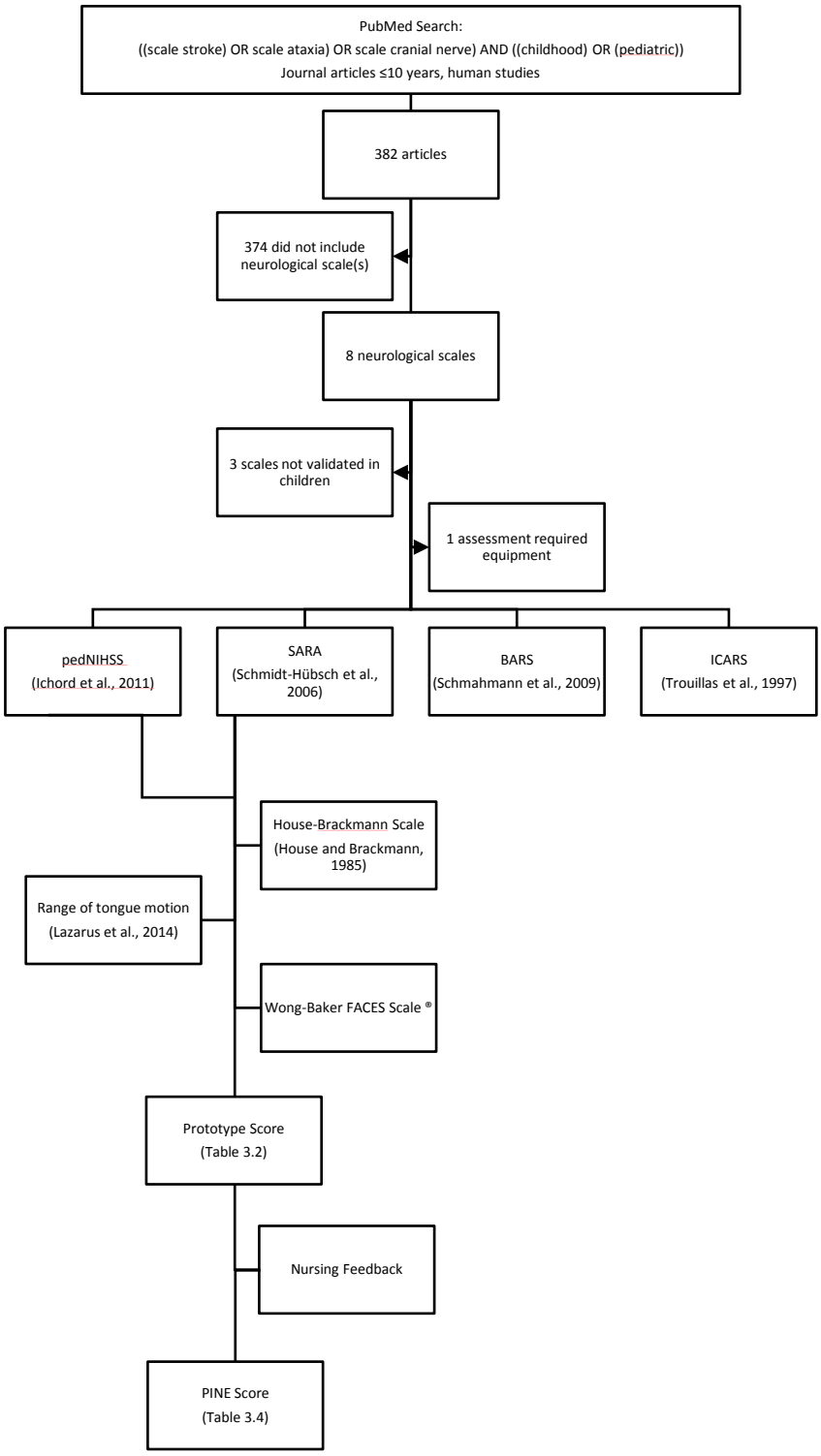

Figure 3.1. Development of the Pontine Infusion Neurological Evaluation Score. A literature search was conducted to identify neurological scales that measure ataxia, long tract signs and cranial neuropathy as cardinal features of diffuse intrinsic pontine glioma. 4 scales were identified. A composite score of the pediatric National institutes of Health Stroke Scale and Scale for Assessment and Rating of Ataxia was generated. Modifications of the House-Brackmann Scale, a tongue range of motion scale and the Wong Baker Scale® were included in a Prototype Score, which was trialled in 20 infusions. The PINE Score was generated from the Prototype Score based on nursing feedback.

The pedNIHSS provided validated items assessing consciousness, eye movement and limb power, which were included in the Prototype Score (Table 3.2). The assessment of facial movement was modified. Assessment of facial palsy using the pedNIHSS is weighted toward assessment of an upper motor neurone pattern of facial weakness, where the upper face is spared owing to bilateral input to the facial nuclei from the cerebral cortices (Figure 3.2). In pontine infusion, facial weakness could arise due to injury to descending corticobulbar fibres or outgoing facial nerve fibres and could also be bilateral.

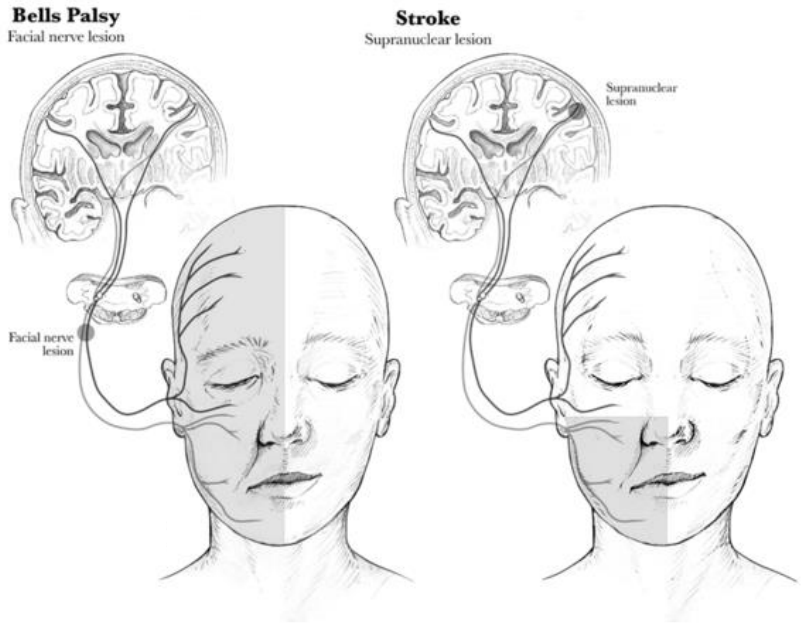

Figure 3.2. Anatomical basis of upper facial sparing in supra-nuclear facial weakness. (Image adapted from Busty A.J. and Kellogg D., 2015, Stroke vs Bell's Palsy: Anatomy image, Evidenced Based Consult, available at: https://www.ebmconsult.com/articles/anatomy-stroke-vs-bells-palsy [Last accessed 7/7/2019])

The House-Brackmann Scale (House and Brackmann et al., 1985) was reviewed as a well-established measure of facial nerve function, which uses gross inspection of the face, at rest and during movement. An advantage of the House-Brackman Scale

is that grading is positively correlated with disfigurement at rest and has been used to treat ophthalmic complications. However, the House-Brackmann Scale can be limited by poor agreement between trained observers, which is attributable to assessment of concurrent involuntary muscle contraction during voluntary movement, known as synkinesis (Coulson et al., 2005). The item for facial movement in the prototype Score was replaced with a simplified version of the House-Brackmann Scale (Table 3.2).

The pedNIHSS also quantifies ataxia by assessment of limb movement. This is elicited using heel-shin slide and finger-to-nose testing. It is scored a maximum of 2 points based on whether it is present in one or two or more limbs. This simplicity is advantageous when designing a scale to be used by non-experts. But, with only two points allocated, it does not award much weight to limb ataxia as a clinical sign. On the other hand, SARA, BARS and ICARS allocate 16, 8 and 52 points for limb ataxia respectively depending on its severity. Although more complex, the higher weighting for limb ataxia may better reflect its impact on functional outcome. On this basis, the simple examination technique and scoring principles of the pedNIHSS were kept but a point was allocated for each individual limb affected, which increases the weighting of ataxia in the overall score.

SARA, ICARS and BARS also quantify ataxia by examining eye movements and dysarthria. Items assessing ataxia using eye movements (i.e. nystagmus) were excluded due to the possible contribution of concomitant oculomotor, trochlear or abducens nerve palsies that could occur during pontine infusion. Dysarthria is common in pontine infusion, recorded in 15% of patients by Souweidane et al., 2019.

Further still, monitoring the ability to communicate is important when designing a score to monitor signs and symptoms of pontine infusion because of the risk of locked-in syndrome (Masuzawa et al., 1993). SARA, ICARS and BARS all score dysarthria by assessing normal conversation. Importantly, because the score is intended to monitor patients at regular intervals throughout infusions, assessing normal conversation could be limited by several factors such as distress of the patient, altered compliance during a long infusion or sleep. The adult NIHSS assesses dysarthria by assessing the repetition of *mama*, *fifty-fifty*, *tip-top*, *baseball player*, *huckleberry* and *thanks* (Brott et al., 1989). This technique was adopted using the SARA scoring criteria.

The Prototype Score (Table 3.2) was completed after inclusion of additional items to assess steroid administration and analgesia - which could have ameliorated the clinical effects of pontine infusion- tongue movement and headache. A tongue movement item was repurposed from a scale used in oral cancer patients to assess range of tongue motion (Lazarus et al. 2014) because none of the scores included in the literature review assessed tongue movement directly. An item for headache utilised the Wong-Baker FACES® Scale, which was already being used in the department.

The Prototype Score (Table 3.2) was trialled in 20 pontine infusions. All examinations were performed with the patient in bed semi-recumbent with the bed at 45°. The Timed Up and Go (TUG) Test, as a validated timed walking test to measure mobility in children with neurological disease (Carey et al. 2016). Nurses who had received training in the use of the Prototype Score were given a paper survey (Table 3.3). Based on nursing feedback, the Prototype Score refined into the Pontine Infusion Neurological Evaluation (PINE) Score (Table 3.4). The PINEScore was taught to PICU nurses during weekly seminars and were supported during infusions with bedside teaching.

Reliability of the PINE Score

Pontine infusions were supervised by a PINEScore-trained nurse. During each infusion, heart rate, blood pressure and respiratory rate were recorded independently by the attending nurse in conjunction with assessment of Glasgow Coma Scale, PINEScore and infusion parameters, including infusion volume and rate of infusion. Nurse-recorded scores were entered directly onto password-protected software, to which the attending doctor did not have access and recorded their assessment independently. Doctor-recorded scores were measured by observing the nurse's assessment. Data acquired from the patient's routine clinical care were used to measure reliability of the PINEscore and quantify neurological and physiological changes occurring during pontine infusion. Doctor-recorded PINEScores were paired with nurse-recorded PINE Scores measured at hour 0 and hour 6 of infusion.

Results

Prototype neurological assessment scale

The Prototype Score was and trialed in 5 patients D, E, F, J and H (see Chapter 2) during 20 infusions. Prototype Scores increased during infusion suggesting accumulating neurological signs and symptoms during pontine infusion. Mean Prototype Score was 5.4 and 6.7 at the beginning and end of infusion respectively, which reached statistical significance (p-value 0.01). Increase in Prototype Score was associated with impaired mobility measured using the TUG test. Mean TUG time at the beginning and end of infusion was 7.6 and 11.2 seconds respectively, which also differed with statistical significance (p-value>0.001). Change in prototype score and change in TUG Time were positively correlated with a Pearson Correlation Co-efficient of 0.267 (p-value >0.001). This suggested that the Prototype Score was a valid and sensitive tool for assessing neurological change during pontine infusion.

Overall, 6/10 nurses agreed that conventional assessment including cardiorespiratory observation and Glasgow Coma Scale testing was helpful during pontine infusion. However, 9/10 either strongly agreed or agreed that the prototype assessment provided useful information during pontine infusion and 8/10 agreed or strongly agreed it could be easily performed with their existing workload. 6/10 nurses said assessment took 1-2 minutes and 9/10 of nurses felt confident or very confident using the assessment. 7/10 nurses reported that the assessment caused distress at the time of assessment; this was reported as occasionally mild distress by 3/7 nurses. All nurses reported that assessment caused no distress or only mild occasional distress in parents. (Figure 3.4). Particular comments included:

"sometimes the children are distressed during infusion-there is no part in the score for this"

"it is confusing about [sic] when we score for giving pain killers"

"sometimes the children are weak in an arm or leg but the score stays the same"

"I had a patient complain of tingling in their face and arms, I don't know how to score it"

"not sure how to assess eye movements"

"the children try hard to repeat the words, but sometimes they slur their speech when they aren't concentrating"

Based on experience and feedback from the Prototype Score, the Pontine Infusion Neurological Evaluation (PINE) Score was compiled as a composite score of brainstem dysfunction in patients receiving pontine infusion. Steroid and analgesia items were removed because prescription was based on clinical protocol rather than patient request. Criteria for distress within the Consciousness item were included. Sensory items were included for the face and body. Examination definitions for eye movements were included stipulating that the limbus has to *'buried'* in the medial or lateral epicanthus in the direction of gaze (Figure 3.3). Points for pupillary abnormalities were also included to detect eye signs in patients with total gaze paresis. Extra points for limb drift, i.e. less than 10 cm, were added to reflect minor weakness.

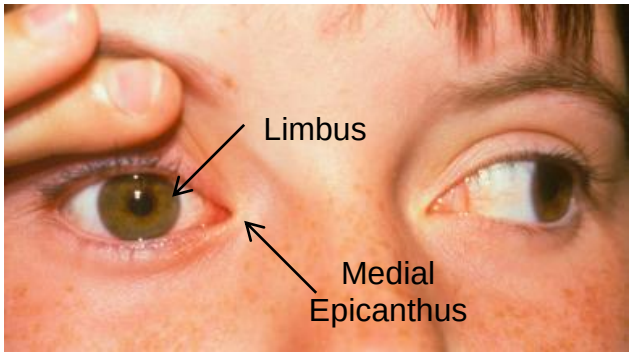

Figure 3.3. Burying the Limbus. Left lateral gaze is elicited demonstrating burying of the limbus in the lateral epicanthus of the left eye. Failure to bury the limbus in the direction of gaze in the right eye demonstrates weakness of the medial rectus. Image modified from Gold, D. Eye Movement Disorders: Conjugate Gaze Abnormalities. In: Liu, Volpe and Galetta's Neuro-Ophthalmology. Third Ed. Diagnosis and Managament (2019) Pages 549-584)

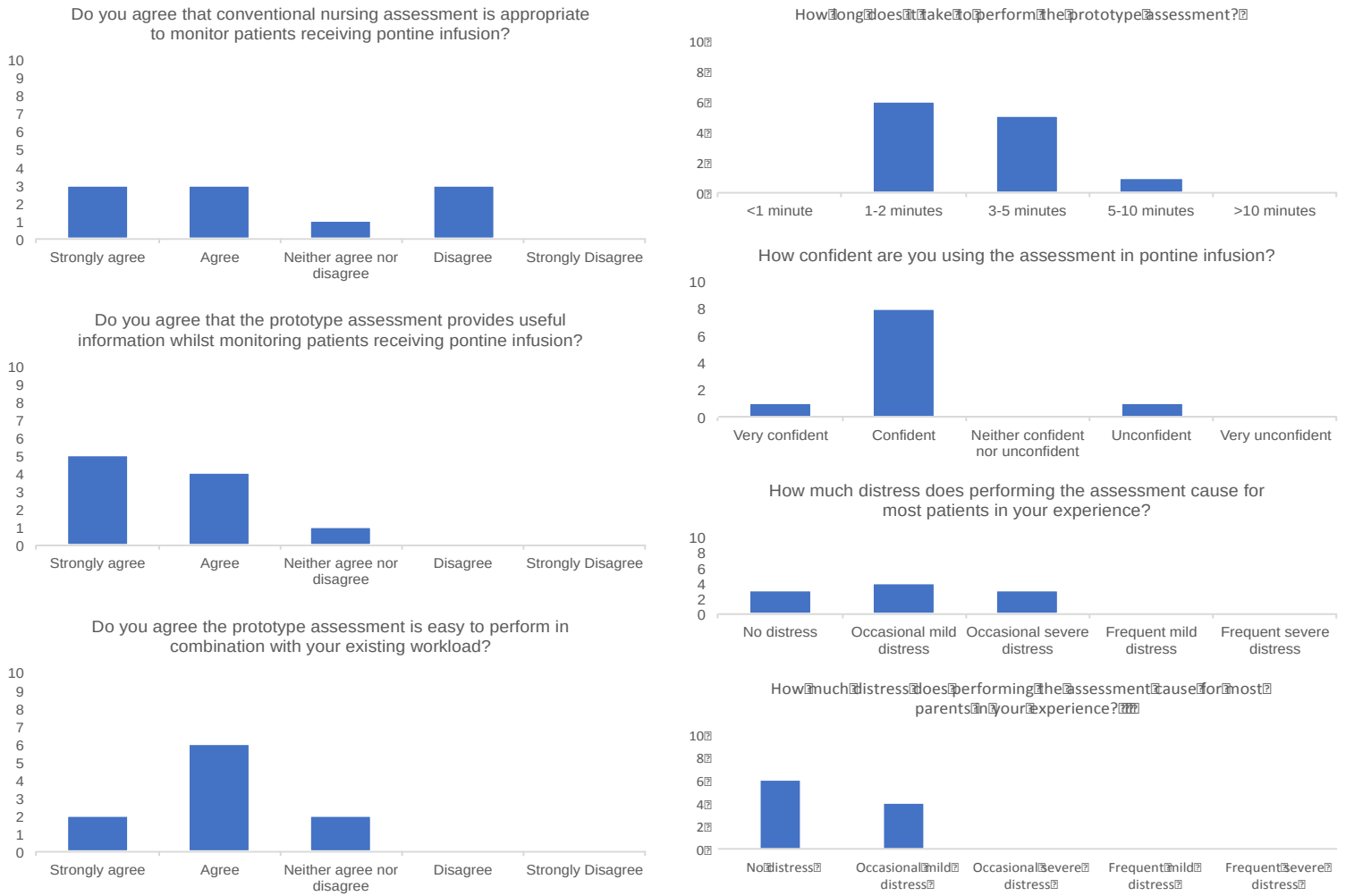

Figure 3.4. Nursing feedback regarding the Prototype Score developed to quantify neurological deterioration during pontine infusion of chemotherapeutics of children with diffuse intrinsic pontine glioma.

Preliminary validity testing of the PINE score

Based on experience and feedback from the Prototype Score, the PINE Score was compiled as a composite score of brainstem dysfunction in patients receiving pontine infusion. Overall 30 pontine infusions in a total of 9 children receiving chemotherapy by pontine CED were analysed. The median age of children treated was 6.6 years (IQR 5.9-6.9). A median of 3.5 (IQR 3-4) infusions were analysed for each patient. Infusions analysed were at different times relative to time of diagnosis (median 6.9 months; IQR 5.9-8.8) and stage of CED treatment (median 2nd cycle of CED; IQR 1.5-3. Each infusion took an average of 7.9 hours (range 5-10 hours) to complete.

Inter-rater reliability testing was performed using paired examinations recorded by the attending doctor and nurse at hours zero and hours six of infusion. Overall, there was strong association between nurse and doctor scores over both time points with a Spearman correlation coefficient of 0.985 (P<0.001). Mean PINE score was 5.49 and 5.5 recorded by nurses and doctor respectively (p-value =0.727). Self-reported items (headache, facial and body sensation) demonstrated 100% agreement. There was absolute agreement between doctors and nurses in 45/60 cases and was within one point in 57/60 cases. The intra-class correlation coefficient (ICC) was 0.98 (95% CI 0.97-0.99; p-value<0.001) with a Chonbach's alpha value of 0.98 and a Kendall's concordance co-efficient of 0.99. Bias in scoring estimated using Bland and Altman methods was very small at 0.03 (Figure 3.5) and disagreement did not demonstrate proportional bias. Also, there was excellent agreement over both time points using weighted kappa calculations. Weighted kappa values were not calculated for scoring of consciousness because all patients were keenly alert at the time of paired assessment. The PINE score was also reliable when both time points were

examined separately. At hour zero, mean PINE Score was 3.9 and 3.8 when recorded by nurses and doctor respectively (p-value=0.184). At hour six, mean PINE score was 7.0 and 7.2 recorded by nurses and doctor respectively (p-value =0.345). ICC at hour zero was 0.99 (95% CI 0.98-0.99; p-value<0.001) with a Chonbach's alpha value of 0.99 and a Kendall's concordance co-efficient of 0.99. ICC at hour six was 0.98 (95% CI 0.93-0.99; p-value<0.001) with a Chonbach's alpha value of 0.98 and a Kendall's concordance co-efficient of 0.97. Weighted kappa values also maintained good concordance at both time points.

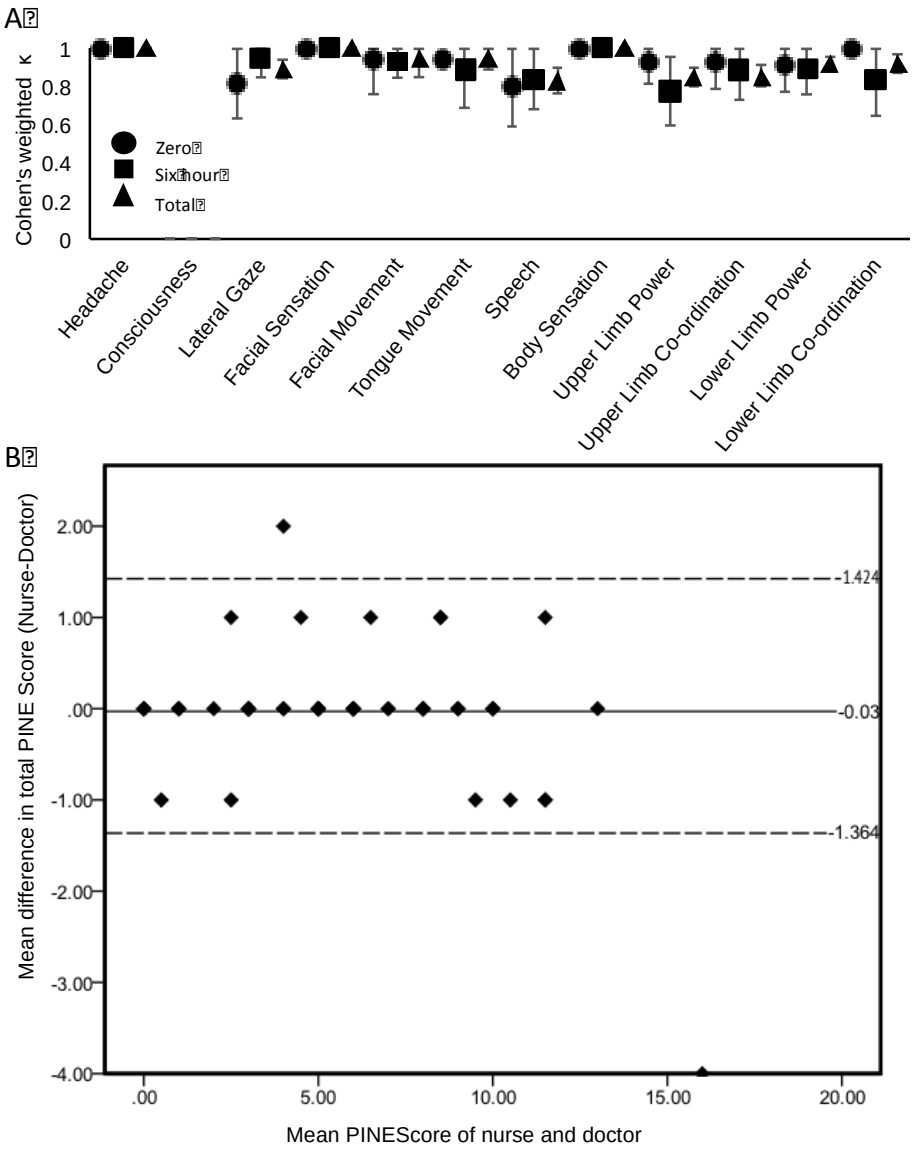

Figure 3.5. Agreement and reliability of the PINEScore. Kappa plots of agreement on PINEScore recorded by nurse and doctor at hour zero and hour six of infusion (A). A Bland Altman Plot of mean difference and mean PINEScore (B)

Discussion

The PINE Score was developed from validated measures of neurological function. It was first trialled as a Prototype Score on patients receiving pontine infusion as an adjunct to conventional monitoring. The prototype score increased after infusion indicating accumulating neurological signs and symptoms, this was correlated with

increasing TUG time suggesting worsening mobility after infusion. Nurses agreed that the prototype was useful; most reported that it took 1-2 minutes and that it was manageable with their existing workload. Based on their feedback, the prototype was finalised into the PINEScore. Using data from 30 infusions at two time points, the PINEScore had excellent inter-rater reliability and there was excellent agreement between items.

Developing and validating a new clinical assessment scale alongside treatment on compassionate grounds is limited in several ways. Awake pontine infusion in DIPG is a treatment limited to a very small number of patients administered by an even smaller group of healthcare professionals. This limits the ability to acquire peer-validity as many experts in paediatric neurology would not have any experience of the treatment. It is likely that if this treatment is taken up in the future the PINEScore may be refined as the wealth of knowledge about awake pontine infusion grows. Moreover, inter-rater reliability was assessed without an experimental protocol using data acquired during routine assessment of the patients. Indeed, the PINEScores were scored simultaneously at the child's bedside as part of their clinical care and it cannot be excluded that unintentional non-verbal communication took place. Examiners were also not blinded to the stage of the infusion therefore it is possible that perceptions about higher risk of neurological disability as infusion progresses may have influenced score interpretation. The PINEScore was only measured in a very small number of children, which is ultimately due to the small numbers of children receiving the treatment. It could be argued that each infusion represents a different neurological phenotype and so the estimates of reliability may be more robust than the small number of children studied would suggest. Nevertheless, the

robustness of reliability estimates would certainly be improved by being tested in patients with and without DIPG in children of different ages. Indeed, a further consideration is how to administer the PINEScore in different languages. DIPG is a rare disease and only a subpopulation are eligible for CED, therefore, patients from different countries are likely to be treated in the same unit. The use of a score to monitor safety of infusion must therefore be validated in a range of languages to ensure safety.

An important issue raised by the nursing staff was the distress caused by the examination. The frequency and severity of procedural anxiety in awake pontine infusion is unknown. Anecdotally, many patients tolerate infusion very well while others demonstrate high degrees of anxiety. Children treated with awake pontine infusion have to face many challenges. The families and children have undergone major life changes in a short space of time; often patients fly long haul flights to receive treatment with the added uncertainty of receiving experimental therapy. All of these could compound their ability to cope. The experience from the child's perspective is difficult to decipher. Reassuringly, it is understood that in clinical situations parents under estimate the health-related quality of life of their children (Upton, 2008). Nevertheless, the reported distress during examination requires further investigation. Ensuring safety of infusion is paramount, and if indeed, systematic examination does adds to the procedural anxiety, it needs to be understood how it can be reduced and whether the benefits are justified. Bearing in mind that no infusion was associated with critical adverse events while using the PINEScore, it may be possible that infusions can be conducted outside of PICU; this could help allay anxiety.

In conclusion, we describe a novel scoring system to that systematically measures neurological signs and symptoms during pontine infusion. Preliminary analysis from data acquired from treatment on compassionate grounds suggests it can be feasibly delivered hourly during infusion. It is reliable and correlates with other measures of neurological function. It is intended that this scoring system will improve the safety of awake pontine infusion and help understand how harm can be avoided in the future. In the absence of a better validated alternative, the PINEScore has become part of the standard operating procedure for awake pontine infusion.

Table 3.1. Individual items from four neurological assessment scales validated in children: the pediatric National Institutes of Health Stroke Scale (pedNIHSS), Scale for Assessment and Rating of Ataxia (SARA), Brief Ataxia Rating Scale (BARS) and International Co-operative Ataxia Rating Scale (ICARS)

Scale	pedNIHSS (points scored)	SARA (points scored)	BARS (points scored)	ICARS (points scored)
Item	Level of consciousness (6) Best Gaze (2) Visual (3) Facial Palsy (3) Motor Arm and leg power (20) Limb ataxia (2) Sensory (2) Best language (3)	Gait (8) Stance (6) Sitting (4) Speech disturbance (6) Finger chase (4) Nose-finger test (4) Fast alternating hand movements (4) Heel-shin slide (4)	Gait (8) Knee-tibia test(4) Finger-to-nose test (4) Abnormalities of ocular pursuit (2) Dysarthria (4) Oculomotor abnormalities (1)	Oculomotor (6) Speech (8) Kinetic (52) Postural (34)

Table 3.2. Prototype Score for assessment of neurological deterioration during pontine infusion

Component and examiner instructions.	Score
Headache Ask: "Do you have a headache? How much does it hurt?" Examiner points to Wong-Baker faces	• No headache (0) • Hurts a little (1) • Hurts little more (2) • Hurts even more (3) • Hurts a whole lot (4) • Worst hurt (5)
Analgesia requirements	• No analgesia given (0) • Analgesia given (1)
Steroids requirements	• No steroids given (0) • Steroids given (1)
Consciousness Look at the patient, which description is most appropriate?	• Keenly alert (0) • Rousable but alert with light stimulation (1) • Rousable with strong stimulation (2) • Reflex movements or unresponsive (3)
Eye movements Ask the patient "Look up and down, left and right" [Ignore nstagmus]	• Full range of eye movements (0) • Abnormal/limited movements in one eye (1) • Abnormal/limited movements in both eyes (2) • No movement in either eye (3)
Facial movements Note the face at rest and on movement "Raise your eyebrows, scrunch up your eyes, blow out your cheeks, show me your teeth"	• Normal (0) • Mild asymmetry apparent on movement only (1) • Unilateral partial paralysis of upper face evident at rest (2) • Total paralysis of upper and lower face on one side or partial paralysis of both side of the face evident at rest (3) • No facial movements (4)
Tongue movements Ask the patient "Stick your tongue out as far as you can"	• Normal range of movement (0) • Deviation with normal protrusion (1) • Unable to protrude tongue beyond lower lip margin (1)
Arm Power The limb is placed in the appropriate position: extend the arms (palms up) at 90˚. Score each arm separately.	• No drift; limb holds 90˚ for full 10 seconds (0) • Drift; limb holds 90˚, but drifts down before full 10 seconds; does not hit bed or other support (1) • Some effort against gravity; limb cannot get to or maintain (if cued) 90˚, drifts down to bed, but has some effort against gravity (2) • No effort against gravity; limb falls (3) • No movement (4)

Table 3.2 continued. Prototype Score for assessment of neurological deterioration during pontine infusion

Limb ataxia The finger-nose-finger test and heel shin test	• Normal co-ordination (0) • Present in one limb (1) • Present in two limbs (2) • Present in three limbs (3) • Present in four limbs (4)
Leg power The limb is placed in the appropriate position: leg raised with leg straight and heel 30 cm off the bed. Maintain for 5 seconds. Score each leg separately.	• No drift; limb held in position for full 5 seconds (0) • Drift; limb held in position but drifts; does not hit bed or other support (1) • Some effort against gravity; limb cannot get to or maintain 30 cm above bed drifts down to bed, but has some effort against gravity (2) • No effort against gravity; limb falls (3) • No movement (4)
Speech Ask the patient to repeat 'huckleberry, mama, fifty-fifty, thanks, baseball, caterpillar'	• Normal • Suggestion of speech disturbance (1) • Impaired speech, but easy to understand (2) • Occasional words difficult to understand (3) • Many words difficult to understand (4) • Only single words understandable (5) • Speech unintelligible / anarthria (6)

Table 3.3. Prototype Score: Nursing Feedback

1. To what extent do you agree conventional nursing assessment is appropriate to safely monitor patents receiving brainstem CED? Please tick the appropriate box				
Strongly agree	Agree	Neither agree not disagree	Disagree	Strongly disagree
2. To what extent do you agree the Prototype Score provides useful information whilst monitoring patients receiving brainstem CED? Please tick the most appropriate box				
Strongly agree	Agree	Neither agree not disagree	Disagree	Strongly disagree
3. To what extent do you agree that the Prototype Score is easy to perform in combination with your existing workload? Please tick the most appropriate box				
Strongly agree	Agree	Neither agree not disagree	Disagree	Strongly disagree
4. How long does the Prototype Score take to perform? Please tick the most appropriate box				
< 1minute	1-2 minutes	3-5 minutes	5-10 minutes	>10 minutes
5. How much distress does performing the assessment cause for most patients in your experience? Please tick the most appropriate box				
No distress	Occasional mild distress	Occasional severe distress	Frequent mild distress	Frequent severe distress
6. How much distress does performing the assessment cause for most parents in your experience? Please tick the most appropriate box				
No distress	Occasional mild distress	Occasional severe distress	Frequent mild distress	Frequent severe distress
7. How confident are you using the assessment in pontine infusion? Please tick the most appropriate box				
Very confident	Confident	Neither confident nor unconfident	Unconfident	Very confident
8. In your opinion what are the best parts of the assessment?				
9. In your opinion what are the 3 things that could be done to improve the assessment?				
10. Any further comments?				

Table 3.4. Pontine Infusion Neurological Evaluation Score

Component and examiner instructions.	Score
Headache Ask: "Do you have a headache? How much does it hurt?" *Examiner points to Wong-Baker faces*	• No Headache (0) • Hurts a little (1) • Hurts little more (2) • Hurts even more (3) • Hurts a whole lot (4) • Worst hurt (5)
Consciousness *Look at the patient, which description is most appropriate?*	• Keenly Alert (0) • Distress (1) • Rousable but alert with light stimulation (2) • Rousable with strong stimulation (3) • Reflex movements or unresponsive (4)
Eye movements *Ask the patient "Look left and right"* *[Ignore nystagmus]*	• Buries the limbus in the epicanthi of the direction of gaze on both sides (0) • Restricted gaze in one eye (1) • Restricted gaze in both eyes (2) • Total gaze paresis with normal pupils (3) • Total gaze paresis with abnormal pupils(4)
Facial Sensation Ask the patient "Do you have numbness or tingling? If so, is it painful? If so, is it severe?".	• No numbness or tingling or pain in the face (0) • Non painful sensory change (1) • Moderately painful sensory change (2) • Severely painful sensory change (3)
Facial movements *Note the face at rest and on movement "Raise your eyebrows, scrunch up your eyes, blow out your cheeks, show me your teeth"*	• Symmetrical face at rest and throughout all movements (0) • Symmetrical at rest with obvious asymmetry during movement involving the lower face only (1) • Symmetrical at rest with obvious asymmetry during movement involving the upper face (2) • Asymmetrical at rest with obvious asymmetry during movement lower face only (3) • Asymmetrical at rest with obvious asymmetry during movement involving the upper face (4) • Barely perceptible or no movement on one side of the face (5) • No movement on either side of the face (6)
Tongue movements *Ask the patient "Stick you tongue out as far as you can"*	• Normal range of movement (0) • Deviation with normal protrusion (1) • Unable to protrude tongue beyond lip margin (1)
Body Sensation Ask: "Do you have numbness of tingling (in the arms legs or body)? If so, is it painful? If so, is it severe?"	• No numbness or tingling or pain in the body or limbs (0) • Non-painful sensory change (1) • Moderately painful sensory change (2) • Severely painful sensory change (3)

Table 3.4 continued. Pontine Infusion Neurological Evaluation Scale

Arm Power The limb is placed in the appropriate position: extend the arms (palms up) 90˚. Drift is scored if the arm falls before 10 seconds. Score each arm separately.	• No drift; limb holds 90˚ for full 10 seconds. (0) • Minor Drift; limb holds 90˚, but drifts down <10 cm or pronates before full 10 seconds; does not hit bed or other support (1) • Drift; limb holds 90˚, but drifts down >10 cm before full 10 seconds; does not hit bed or other support (3) • Some effort against gravity; limb cannot get to or maintain (if cued) 90˚, drifts down to bed, but has some effort against gravity. (3) • No effort against gravity; limb falls. (4) • No movement. (5)
Leg power The limb is placed in the appropriate position: leg straight with heel raised 30 cm above the bed. Maintain for 5 seconds. Score each leg separately.	• No drift; limb held with heel 30 cm off bed for 5 seconds (0) • Drift; limb held above bed but drift less than 10 cm or external rotation at hip (1) • Drift; limb held with heel above bed but drift more than 10 cm; does not hit bed or other support (2) • Some effort against gravity; limb cannot get to or maintain heel at 30 cm above bed; drifts down to bed, but has some effort against gravity (3) • No effort against gravity; limb falls. (4) • No movement (5)
Limb ataxia The finger-nose-finger test and heel shin test	• Normal co-ordination (0) • Present in one limb (1) • Present in two limbs (2) • Present in three limbs (3) • Present in four limbs (4)
Speech Ask the patient to repeat *'huckleberry, mama, fifty-fifty, thanks, baseball, caterpillar'*	• Normal pronunciation and normal conversational speech (0) • Suggestion of speech disturbance in conversational speech only (1) • Suggestion of speech disturbance during pronunciation (2) • Slurring of words (3) • The words are easily understood but there is obvious slurring (4) • Difficult to understand (5) • Noises only (6) • Aphasic (7)

Chapter 4. Chronic intermittent convection enhanced delivery of carboplatin and sodium valproate to the brain stem in children with diffuse intrinsic pontine glioma

Background

The Functional Neurosurgery Research Group has amassed considerable experience of awake pontine infusion following treatment of several patients with DIPG on compassionate grounds using carboplatin and sodium valproate as monotherapies. Carboplatin, as an established anti-neoplastic agent, was used in 5 children with some favourable results. However, carboplatin infusion was associated with side-effects (Singleton et al., 2016) and further patients went onto receive sodium valproate in a hope to reduce the burden of neurological disability from pharmacological toxicity. 10 patients were then recruited for sodium valproate administered by CED. However, switching to sodium valproate monotherapy was still associated with side-effects during infusion and failed to control disease in 4 patients. Preclinical studies show sodium valproate and carboplatin kill DIPG cells synergistically *in vitro* without added toxicity (Killick-Cole et al. 2017). It was hypothesized that the combination of carboplatin and sodium valproate would demonstrate increased efficacy compared to sodium valproate alone and enable reduction in the concentration of sodium valproate. Using a two-day infusion regime, patients were treated as part of a convenience pilot cohort on compassionate grounds. Children with DIPG were infused with combined sodium valproate, the experience from the first 8 patients are presented.

Methods

Ethics and consent

As described in Chapter 2, implantation was approved by the Medicines and Health products Regulatory Authority. Treatment of patients using CED was approved by an institutional ethics committee at Harley Street Clinic Children's Hospital. Parents were consented for the experimental nature of the treatment and the use of their child's information for treatment development and for scientific publication.

Patient selection

Patients D, I and J were initially accepted for treatment with sodium valproate monotherapy and were already implanted with the chronic implantable drug delivery system. 5 patients were accepted for implantation and infusion with combined sodium valproate and carboplatin therapy. Eligibility for treatment and method of drug delivery system implantation has been previously described in Chapter 2.

Pontine Infusions

Carboplatin (0.18 mg/mL) and sodium valproate (14.4 mg/mL) were suspended in 5 mL aCSF in sterile conditions and were supplied in four pre-filled syringes for infusion and connected to the drug delivery system using the transcutaneous bone anchored port (TBAP), as described in Chapter 2. Cycles were performed at 4-6 week intervals. All patients who were implanted for infusion of combined carboplatin and sodium valproate received infusion within 72 hours of drug delivery system implantation. Patients were monitored during infusion by continuous cardiorespiratory monitoring and regular neurological evaluation using the Pontine Infusion Neurological Evaluation (PINE) Score, which was developed specifically for

this purpose (see Chapter 3). The ramping regime used to initiate infusion is described in Chapter 2. Infusion regime was changed from a 4 catheter simultaneous infusion to an infusion performed through transfrontal catheters and transcerebellar catheters on separate days of each cycle. Due to the reduced accumulative flow rate arising from switching to a two-catheter regime, the maximum rate of infusion per catheter was increased upto 0.4 mL/hr if tolerated. Again, infusion was continued to achieve maximum volume of infusion and was limited by onset of significant neurological signs or symptoms. This new infusion regime enabled neurological deficits to be lateralised to specific catheters, e.g. a right cerebellar catheter in the right corticospinal tract would elicit left sided hemiparesis. This was determined using a schema (Figure 4.1). If a deficit could be localised to a specific catheter the flow rate would be reduced by 50%, if the deficit did not resolve the infusion would be stopped. Side-effects that occurred during infusion defined according to Common Terminology Criteria for Adverse Events (CTCAE) (Version 5.0) and whether they persisted for longer than 24 hours or 4 weeks. Recovery of deficit was determined by a combination of clinical examination, review of outpatient notes and parent interview. 3D tumour volume was calculated by outlining tumour boundary on pre-implantation axial T2-weighted slices on neuro│inspire™. Magnetic resonance imaging (MRI) was performed before and after the first infusion of each cycle if tolerated by the patient. Increased hyperintensity on T2* and fluid attenuated inversion recovery (FLAIR) sequences was used as a proxy of CED (Sampson et al. 2007, Tisnado et al. 2016).

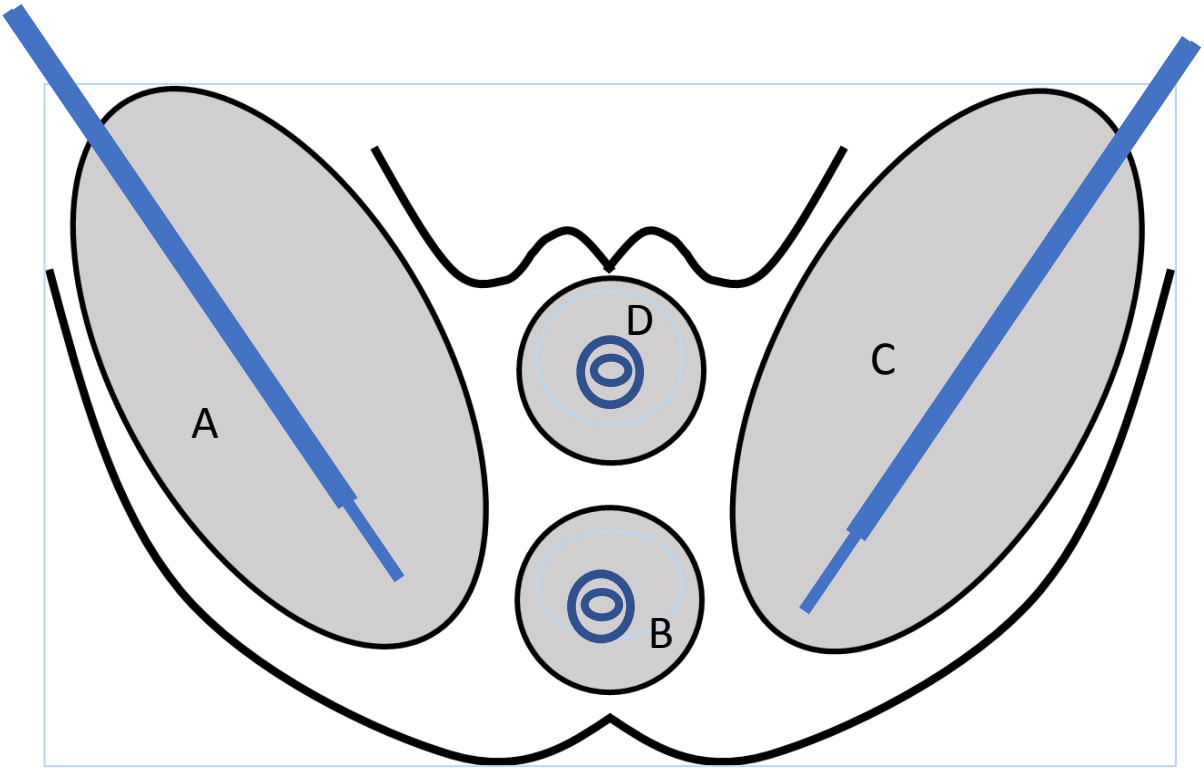

Figure 4.1. Schematic diagram of axial pons with transfrontal and transcerebellar catheters in situ. Side-effects arising from trans-cerebellar catheters (A) and (C) are likely to result in contralateral weakness of the face (with sparing of the upper face), arm or leg, sensory disturbance affecting the face or body or ipsilateral facial weakness (involving upper and lower face) and cerebellar dysfunction. Infusion through the catheters in the dorsal pons (D) could cause headache, ophthalmoplegia, tongue weakness, facial weakness (involving upper and lower face). Infusion through the ventral pons (B) could cause change in sensation in the arms or leg, irritability or quadriparesis. Catheters shown in blue. Expected volume of distribution shown in grey.

Results

8 patients with a median age of 6.6 years at diagnosis (range 3.6-10.7 years) were infused with combined carboplatin and sodium valproate (Table 4.1). Patients K, L and M had biopsy-confirmed World Health Organisation Grade IV tumour with H3 K27M mutations (Louis et al. 2016). The remaining patients had radiological and clinical findings consistent with DIPG. Patients were implanted at a median of 4.6 months (range 3.0-7.8 months) following diagnosis. Mean tumour volume was 16.5

cm^3 (range 7.7-20.2 cm^3). Surgery was well tolerated by all patients. CED infusion commenced within 72 hours of implantation in all 8 cases.

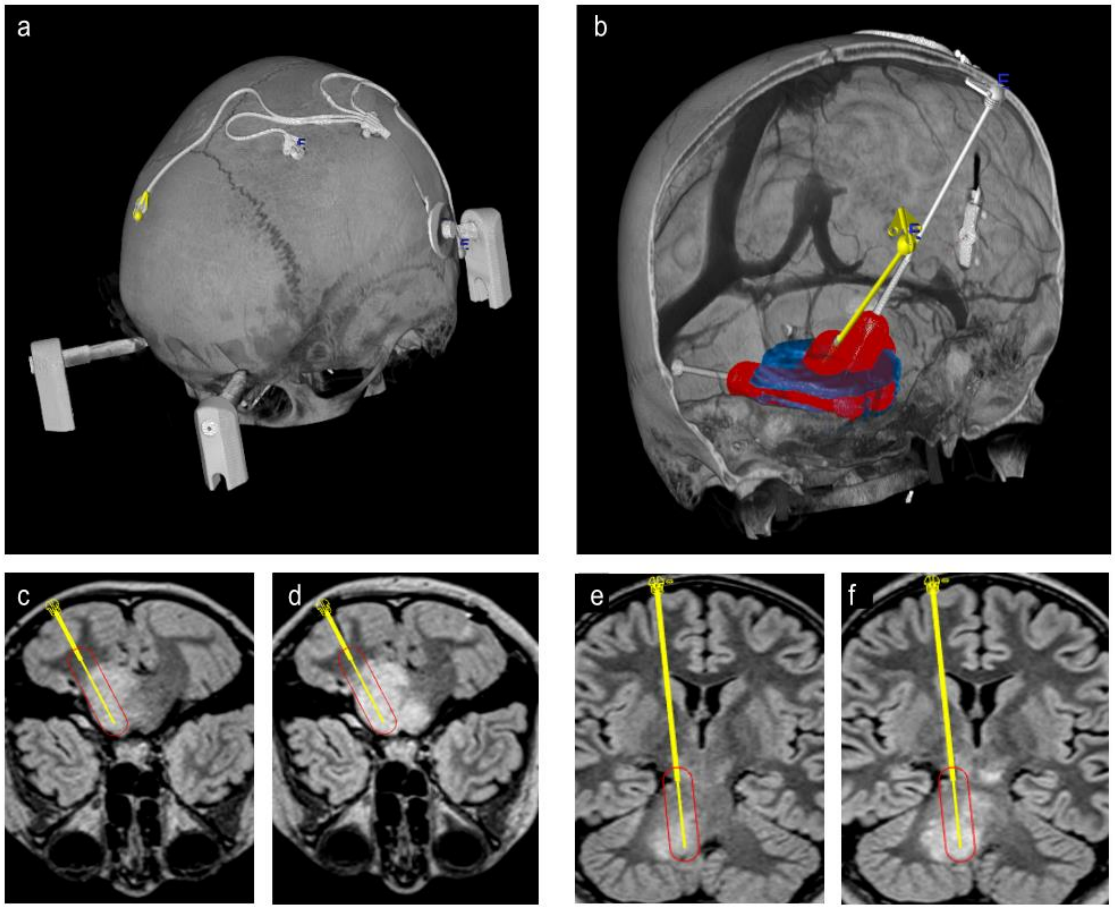

Figure 4.2. Treatment of the diffuse intrinsic pontine glioma using a chronic, implantable drug delivery device. 3D reconstructed computer tomography of a four-catheter drug delivery system is shown in situ (a). Recessed stepped catheters provide controlled reflux (red) along the distal catheter trajectory targeting the tumour (blue) (b). Fluid-attenuated inversion recovery sequences of the left cerebellar and frontal catheters before (c, e) and after (d, f) infusion of 4.1 mL of chemotherapy showing distribution of drug.

36 cycles of combined carboplatin and sodium valproate were administered in total consisting of 55 separate infusions with a median of 3 cycles per patient (range 1-6; Table 4.2) at a median interval of 33 days between cycles (range 23-66 days). 35/36 cycles consisted of two infusions on consecutive days. The second infusion of Patient I's first cycle of combined therapy was contra-indicated by residual facial weakness from the previous day's infusion. Patient I went onto develop progressive disease before her next cycle and received no further CED. Mean infusion time was 7.6 hrs (range 5-11 hrs). High pressures occurred during infusion due to flow impedance within the external system, which was corrected by re-application of the application set where necessary. Post-infusion MRI, when performed, demonstrated increasing pontine volume and increased hyper-intensity consistent with CED in all cases (Figure 4.2). All patients were discharged within 24 hours of finishing infusion. Overall a mean volume of 4.4 mL (range 3.0 mL-5.6 mL) was achieved in each infusion.

Patient D, I and J had previously received other therapy by CED before switching onto combined therapy (Table 4.3). Patient D received 4 cycles (6 infusions) of sodium valproate monotherapy, while Patient I and J had received a cycle of sodium valproate and carboplatin as monotherapy respectively before commencing combined treatment.

Side-effects occurred during every CED infusion; however, no patient suffered severe or life-threatening complications. 62% (34/55) of infusions were associated with headache. Headache was mild to moderate in all but one infusion, which was

relieved by regular analgesia. Headache resolved after stopping the infusion in all cases. Abducens nerve disorders, facial muscle weakness, dysarthria, ataxia, right or left sided weakness were identified in 29% (16/55), 31% (17/55), 29% (16/55), 38% (21/55) and 60% (33/55) infusions respectively. In all cases severity of symptoms were mild - moderate and in most cases these deficits returned to pre-infusion baseline within 24 hours of stopping infusion. However, all patients developed neurological symptoms during CED treatment that persisted for over 24 hours and 6 patients acquired at least one deficit that failed to recover after 4 weeks of follow-up (Table 4.2). Owing to the observed neurological toxicity, the carboplatin concentration was reduced to 0.12 mg/mL due to suspected pharmacological toxicity; this was performed in 6 cycles (Table 2). However, side-effect profiles between cycles using different concentrations of carboplatin were comparable. Similarly, Patients D, I and J who had previously received CED of sodium valproate and/or carboplatin as monotherapies, also experienced similar side-effects during infusion similar to combined therapy. Despite the observed toxicity, patients maintained baseline performance status up to the diagnosis of progression and there were no reports of systemic toxicity associated with combined treatment.

No port failed or became unstable requiring replacement. All patients reported serous exudate around the port. Three patients had confirmed infection around the port but no specific pathogen was isolated. All cases were successfully treated with oral antibiotics. There was no evidence of intra-cranial infection, haemorrhage or cerebrospinal fluid leak arising during or after implantation. Patient L and N were found to have increased hyperintensity within the pons on T2 weighted-imaging after the 3rd and 2nd cycle respectively. This was associated with minimal change in their

clinical phenotype and the radiological changes subsequently improved in Patient L (Figure 4.3) and stabilised in Patient N. This was suggestive an underlying inflammatory process such as pseudo-progression (Carceller et al. 2016).

Median overall survival was 13.9 months (range 8.6-27.0 months) with one patient still alive at time of submission. Tumour in Patient D remained stable after treatment with sodium valproate monotherapy but after treatment with combined therapy there was evidence of reduced tumour signal and volume (Figure 4.3). Patient D and L demonstrated lepto-spinal metastasis and went onto receive palliative radiotherapy. Patient J developed thalamic disease outside of the volume of distribution suggesting local control of disease (Figure 4.3) and went onto receive palliative radiotherapy. Patient M developed extensive cervicothoracic lepto-spinal metastasis, was palliated and died 8.6 months after diagnosis. Patient I and K progressed within the pons dying 13.9 and 8.9 months from diagnosis after receiving 1 infusion (1 cycle) and 4 infusions (2 cycles) of combined therapy by CED.

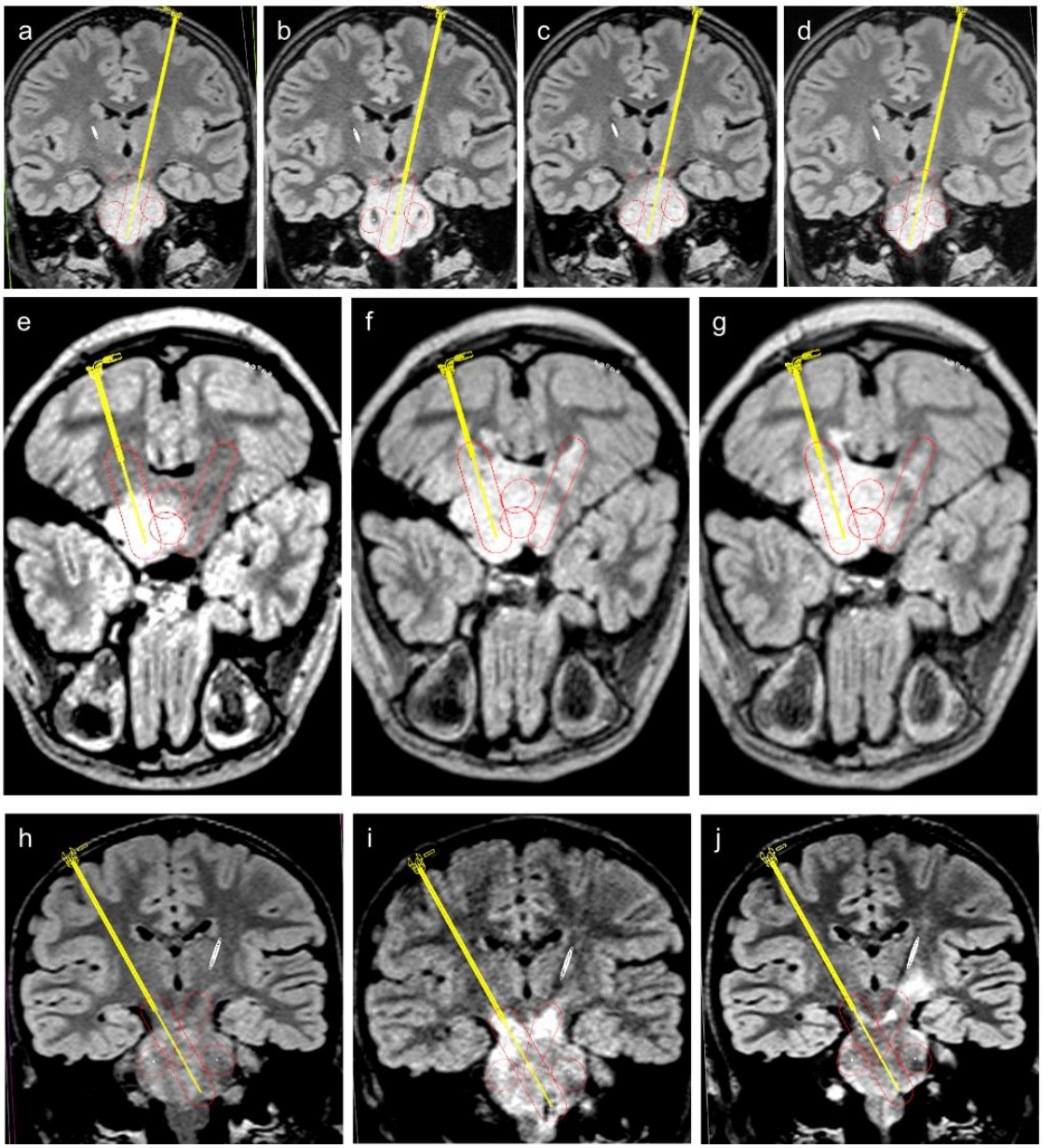

Figure 4.3. Radiological changes in patients receiving pontine infusion of carboplatin and sodium valproate by chronic intermittent convection enhanced delivery (CED) for the treatment of diffuse intrinsic pontine glioma (DIPG). Fluid attenuated inversion recovery (FLAIR) sequences along the left frontal trajectory are shown for Patient B at implantation (a) and after infusion demonstrating drug distribution within the pons (b). After treatment with 4 cycles of sodium valproate monotherapy the tumour was unchanged (d) at 14 months following diagnosis after 5 cycles of combined therapy volume and signal within the pons had reduced (d). FLAIR sequences are shown along the trajectory of the left cerebellar catheter at implantation in Patient L (e), after the third cycle of CED, demonstrating increased intensity within the treatment volume (f) resolving 12 weeks later (g). FLAIR sequences for Patient J along the right frontal trajectory are shown at implantation (h) after infusion demonstrating distribution of drug (i) and development of thalamic disease 9.9 months after diagnosis (j).

Discussion

8 children with DIPG received awake pontine infusion of sodium valproate and carboplatin as combined CED treatment on compassionate grounds. There were encouraging signs of efficacy. Overall patients survived longer than expected for patients with DIPG. There were two objective responses. Progression of disease also occurred outside of the pons in three cases, suggesting that pontine infusion may locally control disease. Implantation of this chronic, implantable drug delivery system was also well tolerated allowing repeated infusions. This experience has identified key challenges in developing awake pontine infusion as a feasible treatment that should be interrogated as part of a clinical trial.

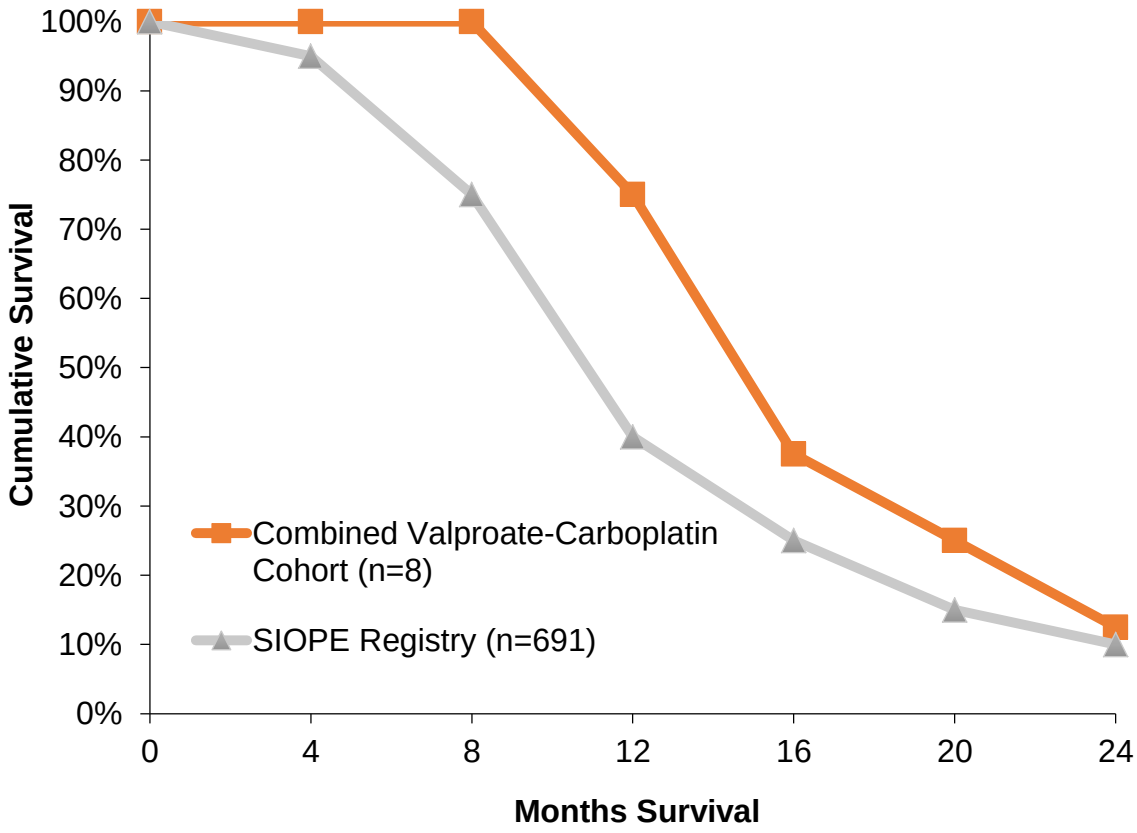

Figure 4.4. Survival curves for 8 children with diffuse intrinsic pontine glioma (DIPG) treated with combined valproate-carboplatin administered by chronic intermittent convection enhanced delivery versus the SIOPE DIPG Registry (Veldhuijzen van Zanten et al., 2017)

There are several important lessons from this experience. It is important to exclude patients who cannot be feasibly treated. Experience from this cohort, demonstrated that infusion volume and the neurological side-effects of CED are important factors for developing eligibility criteria. Using this catheter configuration, disease beyond the pons, the immediately adjacent midbrain and cerebellar peduncles cannot be reached at an infusion volume of 4-5 mL. Similarly, tumour >36 cm^3 would not be covered by a volume of infusion of 9-10mL over 2 days' of infusion. Hence, patients with disease outside the pons and/or >36 cm^3 would be unlikely to be treated successfully. Indeed, considering the side-effects that can arise from pontine infusion, the patient's pre-morbid status should also be carefully evaluated. Patients with poor performance status or severe neurological impairments at baseline may not tolerate CED. As such, eligibility criteria for a future trial should include limits on disease volume, anatomical distribution and clinical status of the patient.

Importantly, pontine disease remained stable in 6 patients who received repeated infusions of carboplatin and sodium valproate. Expansion of radiological disease within the pons predicts poor outcome and progression of DIPG is associated with severe neurological symptoms (Steffen-Smith et al. 2014, Veldhuijzen van Zanten et al. 2016). If CED can control pontine disease locally it could improve quality of life and extend survival. Indeed, two patients had features consistent with pseudo-progression, which is associated with better prognosis (Carceller et al. 2016). The causes of this are unclear and can occur following radiotherapy (Carceller et al. 2016). However, it is possible that local delivery of chemotherapeutics, particularly histone deacetylase inhibitors, may initiate local immune activation.

It is important to weigh potential advantages of local delivery against the expected toxicity of the treatment. Like infusion with carboplatin and sodium valproate as single agents, combined therapy was associated with neurological deficits that were comparable in type and severity to those reported in the trial published by Souweidane, et al., 2018. Reported side effects occurred exclusively during infusion, the cause of which in unclear. Studying these side effects using the PINEScore could elucidate the cause and predictors of such toxicity.

In conclusion, this experience of treating DIPG using combined carboplatin and sodium valproate delivered directly to the pons using a chronic, implantable drug delivery system was feasible, was not associated with treatment-related life-threatening events and demonstrated encouraging signs of efficacy. Weighing the potential benefits of local control of disease versus the impact of pontine infusion on quality of life in DIPG requires study in an appropriately designed clinical trial using validated outcome measures designed to evaluate CED and DIPG.

Table 4.1. Characteristics of 8 patients receiving chronic, intermittent convection enhanced delivery of combined carboplatin and sodium valproate for the treatment of diffuse intrinsic pontine glioma

Patient	Age at diagnosis (years)	Biopsy	Diagnosis to implant	Tumour volume (cm^3)	Number of CED cycles (infusions) of combined therapy	Additional treatment	Survival from diagnosis (months)
D	10.6	-	4.9	17.8	5 (10)**	Post CED surgical resection, immunotherapy and palliative radiotherapy	17.8
I	7.8	-	7.9	16.9	1 (1)**	4 cycles carboplatin	13.9
J	6.9	-	3.3	18.1	4 (8)**	Post CED-palliative radiotherapy	13.5
K	6.3	H3K27M	3.9	20.2	2 (4)	Panobinostat delivered by CED	8.9
L	10.7	H3K27M	4.2	16.2	6 (12)	Post CED- Palliative radiotherapy	20.6
M	6	H3K27M	4.9	15.8	3 (6)	-	8.6
N	3.6	-	3.6	11.7	3 (6)	-	15.2
O	5.7	-	6.5	7.7	4 (8)	3 cycles carboplatin and irinotecan; Post CED - intra-arterial chemotherapy	27.0*

*patient alive at time of submission

Table 4.2. Neurological toxicity occurring during pontine infusion according to Common Terminology Criteria for Adverse Events Version 5.0 in 8 patients with diffuse intrinsic pontine glioma treated with chronic, intermittent convection enhanced delivery of carboplatin and sodium valproate

Patient	Full recovery within 4 weeks (Grade)	Persistent at 4 weeks (Grade)
D	Right-Sided Weakness (1);	Facial muscle weakness (1); Left-Sided Weakness (2); Abducens nerve disorder (1)
I	Facial muscle weakness (1); Left-sided weakness (1); Ataxia (1); Dysarthria (1)	-
J	Ataxia (1)	Right-sided weakness (2)
K	Ataxia (2)	Abducens nerve disorder (1); Facial muscle weakness (2); Left-sided weakness (2)
L	Abducens nerve disorder (1)	Left-sided weakness (1)
M	Ataxia (1)	-
N	Abducens nerve disorder (1); Ataxia (1); Dysarthria (1); Right-sided weakness (1)	Facial muscle weakness (2); Left-sided weakness (1)
O	-	Facial muscle weakness (1); Abducens nerve disorder (2)

Table 4.3. Treatment schedule of 8 patients with diffuse intrinsic pontine glioma treated with combined sodium valproate (VA) and carboplatin (C) administered by chronic intermittent convection enhanced delivery

Patient		Cycle 1		Cycle 2		Cycle 3		Cycle 4		Cycle 5		Cycle 6		Cycle 7		Cycle 8		Cycle 9		
D	Days from implant	1		29		57		85		118		141		169		214		247		Lepto-spinal metastasis 9.5 months after CED
	Drug	VA		VA		VA		VA		C+VA		C+VA		C+VA		C+VA		C+VA		
	Infusion day	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
	Volume (mL)	3.4	3.8	4.1	4.8	4.0	-	3.6	-	4.8	2.9	4.7	3.1	4.7	4.5	4.2	3.3	4.1	4.1	
I	Days from implant	1		67		99														
	Drug	VA		C		C+VA		Pontine progression at 5.2 months after CED				-		-		-		-		-
	Infusion day	1	2	1	2	1	2													
	Volume (mL)	4.0	-	4.8	2.0	3.7	-													
J	Days from implant	1		31		58		85		130		163								
	Drug	VA		C 0.18		C+VA		C+VA		C+VA		C+VA		Thalamic progression at 6.6 months after CED				-		-
	Infusion day	1	2	1	2	1	2	1	2	1	2	1	2							
	Volume (mL)	6.0	2.8	4.6	4.8	4.3	4.1	4.5	4.6	4.7	4.0	4.7	4.0							
K	Days from implant	1		36																
	Drug	C+VA		C+VA		Pontine progression at 2.7 months after CED				-		-		-		-		-		-
	Infusion day	1	2	1	2															
	Volume (mL)	5.0	3.6	5.2	4.7															

Table 4.3 continued. Treatment schedule of 8 patients with diffuse intrinsic pontine glioma treated with combined valproic acid and carboplatin administered by chronic intermittent convection enhanced delivery

Patient		Cycle 1		Cycle 2		Cycle 3		Cycle 4		Cycle 5		Cycle 6		Cycle 7	Cycle 8	Cycle 9	
L	Days from implant	1		29		60		114		156		200					
	Drug	C+VA		C+VA		C+VA		C+VA		*C+VA		*C+VA					
	Infusion day	1	2	1	2	1	2	1	2	1	2	1	2	Died 20.6 months following diagnosis		-	-
	Volume (mL)	4.2	4.1	4.6	3.9	4.5	5.0	4.2	3.8	4.9	4.0	3.9	4.0				
M	Days from implant	3		41		73											
	Drug	C+VA		C+VA		C+VA		Lepto-spinal metastasis at 2.7 months after CED				-		-	-	-	-
	Infusion day	1	2	1	2	1	2										
	Volume (mL)	5.0	5.6	4.3	5.2	4.1	4.4										
N	Days from implant	2		53		108											
	Drug	C+VA		*C+VA		*C+VA		Died 15.2 months following diagnosis				-		-	-	-	-
	Infusion day	1	2	1	2	1	2										
	Volume (mL)	5.2	4.0	4.1	4.1	4.5	4.3										
O	Days from implant	2		29		64		100									
	Drug	C+VA		C+VA		*C+VA		*C+VA		Alive 23.0 months from diagnosis				-	-	-	-
	Infusion day	1	2	1	2	1	2	1	2								
	Volume (mL)	5.1	4.0	4.2	4.4	4.2	4.4	4.1	4.0								

Valproic acid (VA); carboplatin at 0.18mg/ml (C); combined carboplatin at 0.18mg/ml with valproic acid at 14.4mg/ml (C+VA); combined carboplatin at 0.12mg/ml with valproic acid at 14.4mg/ml (*C+VA)

Chapter 5. Clinical predictors of infusion-related toxicity during pontine infusion in children with diffuse intrinsic pontine glioma using a novel neurological assessment scale

Background

Pontine infusion of chemotherapeutics in patients with diffuse intrinsic pontine glioma (DIPG) is associated with neurological side-effects (Souweidane et al. 2018, Singleton et al. 2016). Being able to understand the neurological changes that take place during pontine infusion and how to prevent long-term side effects is an important step in the translation of this new therapy. The benefit of the chronic, implantable drug delivery system compared to other devices is that patients can be infused awake. Apart from being able to avoid repeated general anaesthetic, this also provides the clinician with continuous feedback about the integrity of vital neural structures throughout infusion.

It is well understood that clinical observations are useful predictors of patient deterioration. Amalgamation of physiological parameters such a heart and respiratory rate, arterial blood pressure and oxygen saturations into early warning scores (EWS) are vital tools in identifying the unwell patients, guiding intervention and reducing harm (Downey et al. 2017). Patients with DIPG receiving infusion of chemotherapeutics directly into the pons are at risk of critical deterioration from various foreseeable causes including hydrocephalus, brainstem haemorrhage and ischaemic stroke. Indeed, given the experimental nature of the treatment and the limited understanding of the pharmacodynamics of direct intra-parenchymal and intra-tumoural drug administration, some risks may be unforeseeable. As such, patients receiving pontine infusion reside in a critical neurological state and

developing method of quantifying and predicting deterioration, akin to the EWS, would be helpful. Chapter 3 describes the development and preliminary validation of the Pontine Infusion Neurological Evaluation Score (PINEScore), which was designed to measure the complex neurological changes taking place in children with DIPG receiving pontine infusion. We hypothesized that neurological changes taking place during the infusion can be used to identify risk factors for persistent neurological side effects.

Methods

Data from 55 pontine infusions in 8 children with DIPG as described in Chapter 4 were analysed. PINEScores were measured by the attending nursing staff before each infusion commenced and at every hour during infusion until the end. If children remained on Paediatric Intensive Care Unit (PICU) after the infusion had finished, PINEScores were recorded until they were discharged to the ward. PINEScores were inputted directly onto a computer database using critical care and anaesthesia information software, ICIP® (Phillips, Surrey), together with the infusion rates and volumes per catheter, Glasgow Coma Score and cardiorespiratory parameters.

Individual items of the PINEScores (i.e. headache, consciousness, ophthalmoplegia etc) were recorded on to a database for analysis using Microsoft ® Excel together with infusion rates and volumes of infusion. Statistical Package for Social Science (Version 23; IBM, USA) was used for statistical analysis. To accommodate for differing patient status at baseline, PINEScore were analysed in two ways: firstly, by total PINEScore and secondly by change in PINE score from pre-infusion baseline (ΔPINE). Infusion-related side effects were identified using the PINEScore by

changes from pre-infusion baseline. Comparisons between PINEScores were performed using t-test calculation. Correlation calculations were calculated using Spearman's correlation co-efficient. Frequency of side-effects was determined at the time of onset. Persistent deficits were defined as changes in PINEScore from pre-infusion baseline that failed to recover after 24 hours, all other deficits were defined as transient. Resolution of side effects was evaluated by parent and patient interview, review of outpatient letters and clinical examination. Risk of persistent deficits were described using odds ratios (OR). Analysis of volume of infusion was performed after volumes had been rounded to the nearest integer. Statistical significance was defined as p-value <0.05.

Results

55 infusions of carboplatin combined with sodium valproate were performed in 8 children (3-11 years) as described in Chapter 4. Median PINEScore at the start of each infusion was 2 (range 0-16). Pre-infusion baseline PINEScores demonstrated weak (0.45) and moderate (0.63) correlations with numbers of infusions administered and days since diagnosis respectively using Spearman correlation co-efficient calculations, both of which reached statistical significance (p-value<0.001). Changes in PINEScore demonstrated the accumulation of neurological and signs and symptoms during infusion (Figure 5.1.)

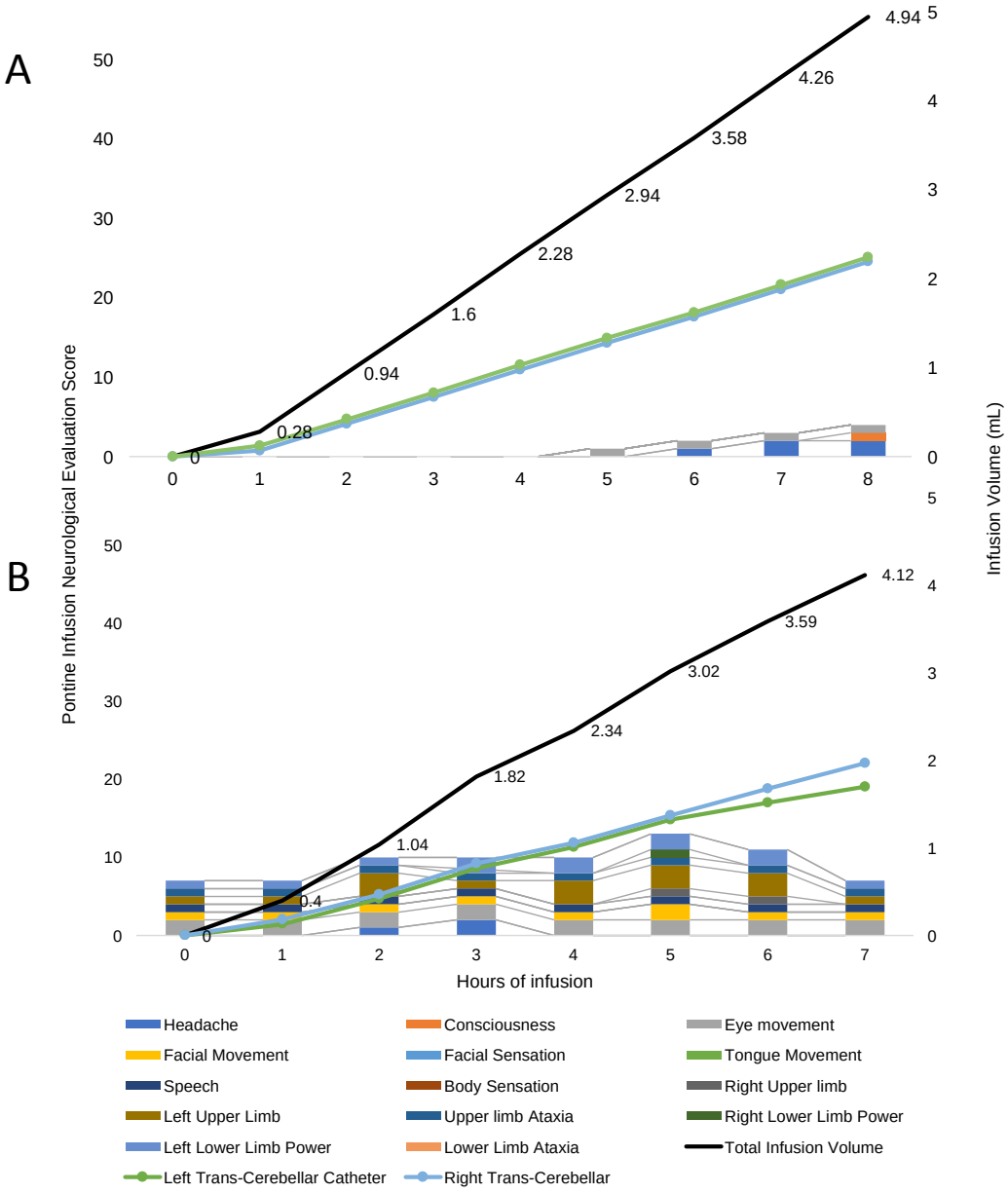

Figure 5.1. Neurological signs and symptoms identified using the Pontine Infusion Neurological Evaluation Score (PINEScore) during pontine infusion in two children with diffuse intrinsic pontine glioma (DIPG). Representative graphs of neurological signs and symptoms identified using the PINEScore are presented for two children with DIPG during pontine infusion performed through two transcerebellar catheters. PINEScores (primary y-axis) per item are presented as stacked bar charts, with each colour representing a different neurological sign or symptom versus the hour of infusion (x-axis). Infusion volumes are presented in total (black line) and in the left (blue) and right (green) transcerebellar catheters (secondary y-axis) versus hour of infusion (x-axis). Graph A demonstrates an untitrated infusion where the infusion is continued despite increase in PINEScore. Graph B demonstrates the reduction in flow rate of the left frontal catheter at onset of increased scores pertaining to right upper and lower limb weakness; by the end of infusion, scores for the right upper and lower limb have reached pre-infusion baseline.

Mean PINEScore increased during infusion from 3.3 to 5.7, which differed with statistical significance (p-value >0.001; Figure 5.2). Mean ∆PINE increased with infusion and infusion volume demonstrated weak positive correlation (0.437) with ∆PINE; this reached statistical significance using (p-value<0.001). There was no difference between mean PINEScore before trans-frontal and trans-cerebellar infusions at 3.8 and 4.0 respectively (p-value =0.89). Mean ∆PINE at the end of infusion, however, was greater during trans-cerebellar infusions than trans-frontal infusions at 2.45 and 1.67 respectively, although this did not reach statistical significance.

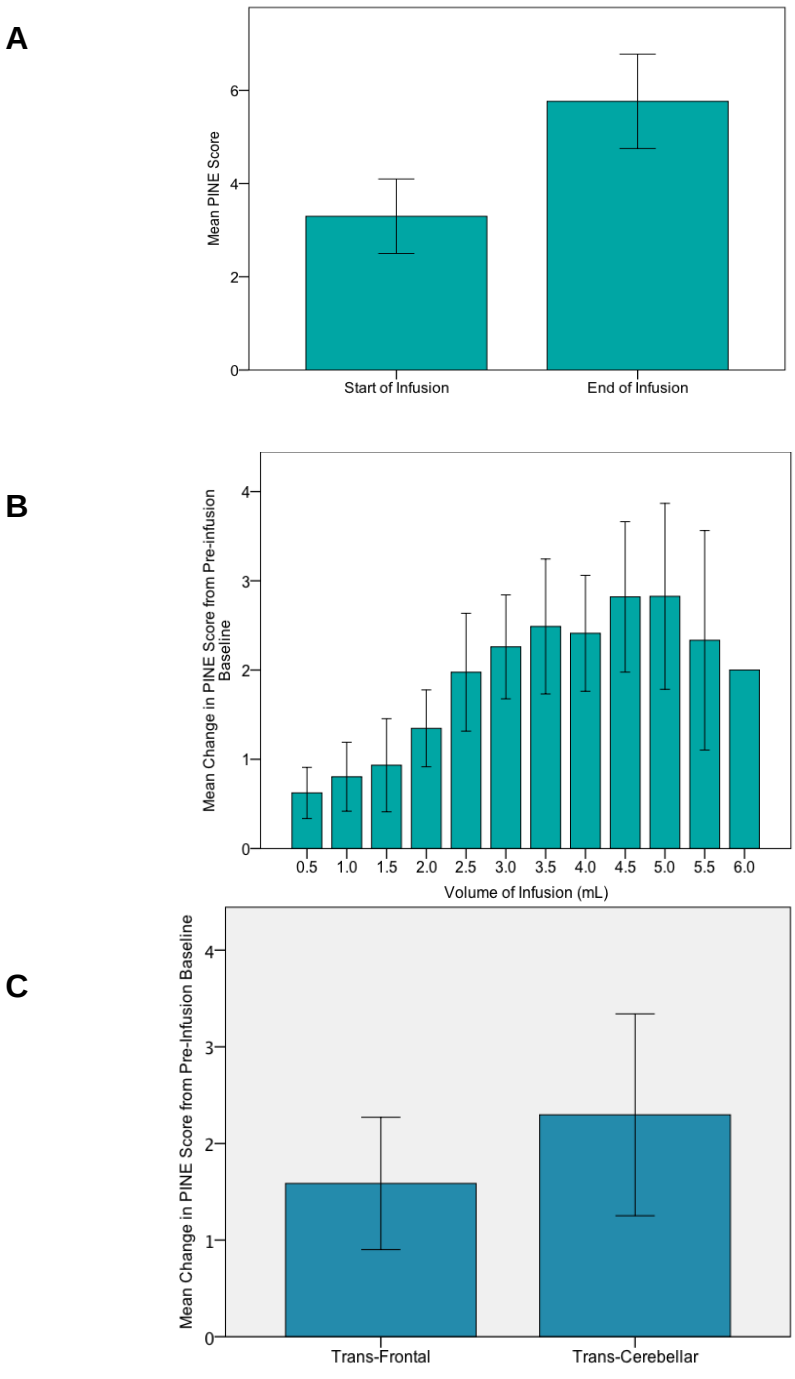

Figure 5.2. Changes in neurological signs and symptoms measured using the Pontine Infusion Neurological Evaluation Score (PINEScore) in 55 pontine infusions in 8 children with diffuse intrinsic pontine glioma. A. Mean PINEScore increased from 3.29 to 5.65, which reached statistical significance (p-value <0.000); B. Mean change in PINEScore increases with volume of infusion; C. Mean change in PINEScore is higher during transcerebellar infusion than trans-frontal infusion but this does not reach statistical significance: Error bars 95% Confidence Interval

PINEScores identified 157 symptoms during 55 pontine infusions, the most common of were headache (33/157) and limb weakness (49/157) (Table 5.1.). According to the Wong Baker Faces Scale, headache was reported as hurting ‘a little’, ‘a little more’, ‘even more’ and ‘a whole lot’ in 17, 11, 4 and 1 cases respectively. 74 and 50 transient and persistent neurological deficits were identified respectively. 18 of the persistent deficits were present at 4 weeks’ follow-up (Table 5.1). At the onset of deficit, the attending physician must weigh up the benefits of continuing infusion versus the immediate risk to the patient and the likelihood of the deficit becoming persistent. In this cohort, no deficits were an immediate risk to the patient; however, 50/124 deficits became persistent and 18 failed to recover by 4 weeks. Predicting non-recoverable deficits is central to managing pontine infusion safely. The ratios of transient and persistent deficits were calculated according to duration of deficits during infusion, the occurrence of the deficit during previous infusions, the maximum severity measured using the PINEScore and volume of infusion (Table 5.2.). The risk of an observed deficit becoming persistent was calculated using binary logistic regression (Figure 5.3.). Deficits that had occurred during a previous infusion and those that occurred during a trans-cerebellar infusion were more likely to be persistent with OR 2.333 (95% CI 1.094-4.976; p-value =0.028) and 2.155 (1.029-4.513; p-value=0.042) respectively. Similarly, if an infusion was stopped or titrated rather than continued, the deficit was less likely to be persistent, OR 0.473 (95% CI 0.177-0.948; p-value=0.037). Other factors such as maximum severity of deficit (indicated by increase in PINEScore greater than 1) and duration of deficit during infusion longer than 3 hours trended toward increased risk of persistent deficit but failed to reach statistical significance. Deficits acquired after 3 mL of infusion tended

toward reduced risk of becoming persistent; however, they did not reach statistical significance (Figure 5.3.).

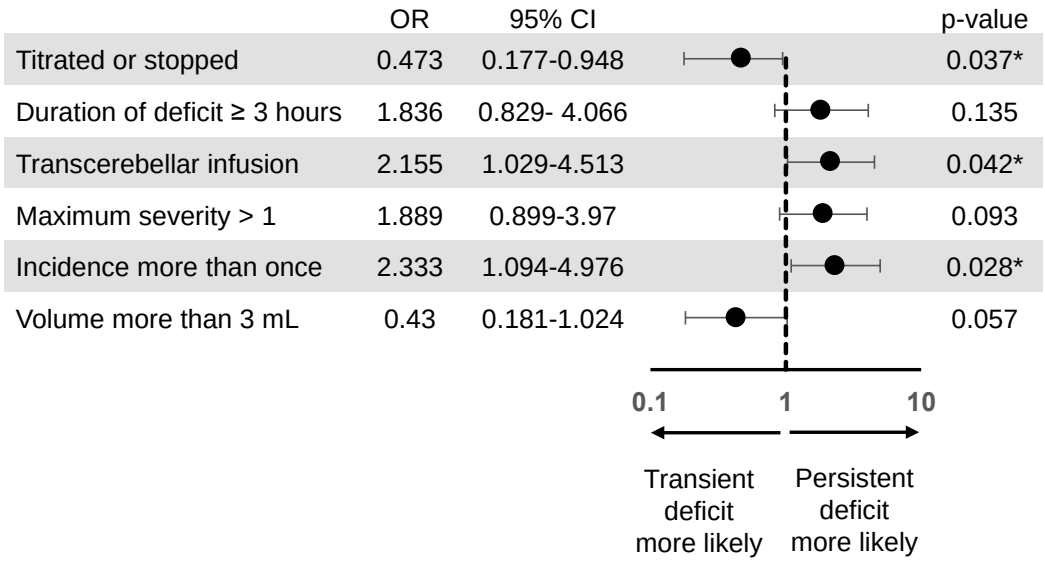

Figure 5.3. Risk factors for persistent deficits arising from 55 pontine infusions in 8 children with DIPG. A forest plot demonstrating unadjusted odds ratios for risk of an infusion-related deficit becoming persistent.

After 8 infusions in 5 children (Patients D, I, J, L and M) PINEScores were recorded while the patient remained in PICU after the infusion had finished (Figure 5.4). This enabled quantification of recovery following infusion using the PINEScore. Mean total PINEScore and mean ΔPINE at the end of infusion was 8.6 and 6 respectively. After a mean recovery time of 1.5 hours total PINE Score and ΔPINE decreased to 5.6 and 3.4 respectively; however, only changes in ΔPINE score reached statistical significance (p-value=0.035). When scores pertaining to headache were excluded these results were no longer statistically significant.

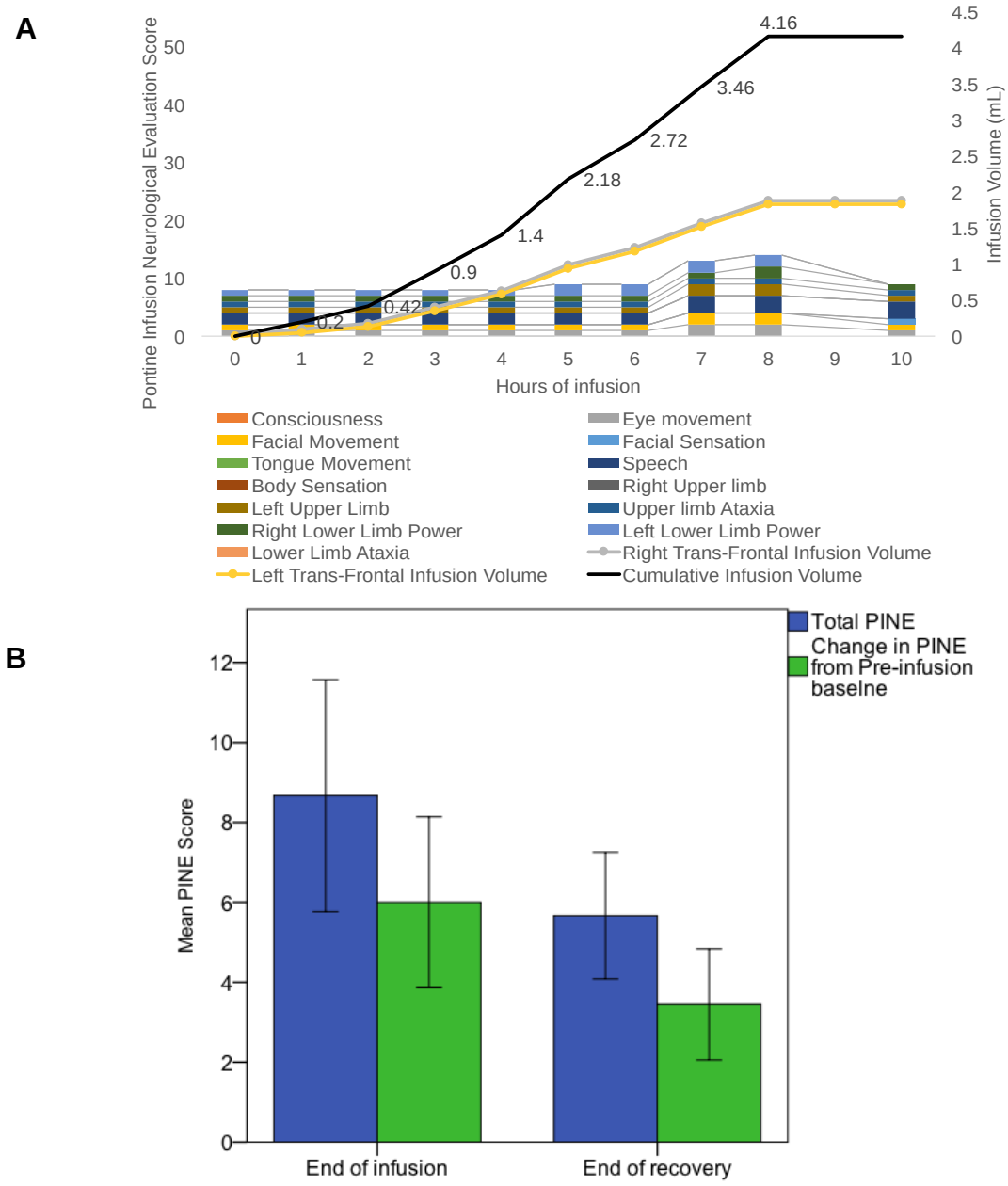

Figure 5.4. Recovery of neurological signs and symptoms using the Pontine Infusion Neurological Evaluation Score (PINEScore) after end of pontine infusion in patients with diffuse intrinsic pontine glioma (DIPG) A. A representative graph demonstrating reduction in PINEScore following cessation of infusion in a patient with DIPG. B. Mean PINEScore and change in PINE Score from pre-infusion baseline at the end of infusion and at the end of recovery. Error Bars 95% confidence intervals.

Discussion

Neurological signs and symptoms in 8 children during 55 pontine infusions of combined carboplatin and sodium valproate were systematically monitored using the PINEScore. The PINEScore increased during pontine infusion reflecting the accumulation of neurological signs and symptoms during infusion. Headache was the most common symptom. All patients developed an infusion-related deficit, the most common of which was limb weakness and nearly half of deficits went on to be persistent. This is the first time the neurological deterioration during pontine infusion has been quantified and represents an avenue for improving its safety. Data derived from the PINEScore suggests that deficits have occurred previously during an infusion or occur during a trans-cerebellar infusion are more likely to be persistent and that the risk of a deficit becoming persistent is less likely when the infusion is either stopped or slowed immediately. Other factors that may be associated with neurological recovery include the severity and duration of deficits during infusion.

There are several caveats to this analysis. This data is derived from a scoring system that has not been rigorously validated and its normative values are unknown. It has been used outside an experimental protocol and the recovery of deficits were defined retrospectively. Nursing staff were unblinded to the infusion volumes and the titrations. It is possible that PINEScores may be biased by preconceived ideas about how patients respond during infusion. Furthermore, intra-rater variability of the PINEScore is unknown and therefore may not be reliable when quantifying recovery or deterioration between infusions. There is also no understanding of what change in

PINEScore is clinically significant. The addition of a time point to record PINE Scores after infusion would also help quantify recovery.

Despite these limitations, it is essential that the safety of CED in DIPG is improved and the impact of treatment on the quality of life is reduced. Bearing in mind conventional neurological monitoring is insensitive to many of the signs and symptoms provoked by pontine infusion, it is important that we learn as much as possible from this preliminary experience before the treatment is extended into a clinical trial. It would appear that the neurological injury during pontine infusion is accumulative. Total PINEScore was positively correlated with the number of infusions. The risk of a deficit being persistent was also increased if it had occurred during a prior infusion. Admittedly, this could be confounded by progression of disease because deficit incidence and number of infusions are also co-correlated with time from diagnosis. However, considering that these infusions were performed prior to diagnosis of clinical and radiological progression it is likely that the infusion is still an important contributory factor to the accumulation of neurological deficits over time. Therefore, reducing the frequency of neurological side-effects during infusion would be advantageous.

It is evident that 30% of the recorded deficits occurred <1mL infusion. Interestingly, deficits occurring at the end of infusion were less likely to become persistent. It would appear that risk of persistent side-effects are not dependent on volume of infusion. This is encouraging because we may still be able to maximise volume of infusion to treat the peri-tumoural brain without necessarily exposing the child to more risk. The risk of persistent side-effects was increased if the infusion was

continued rather than stopped or reduced and tended toward greater risk of persistence if the infusion was continued for 3 or more hours after deficit onset. This would suggest that the way in which the infusion is conducted is a key determinant of treatment-related morbidity. Responsive and prompt infusion adjustment may help to improve the safety profile. *Time is Brain* is a mantra of modern stroke medicine, emphasizing the time-sensitive therapeutic window to relieve brain ischaemia and reduce brain injury (Hill and Hachinski 1998). This evidence suggests that this principle may also apply to pontine infusion, immediately stopping or reducing the infusion into the pons may reduce the risk of long-term deficits - presumably due to the relief of pressure on eloquent neural structures.

In the early experience of using carboplatin and sodium valproate as monotherapies, it was thought that neurological side-effects were difficult to avoid in pontine infusion. However, these data suggest that if the risk factors for persistent deficits can be avoided the accumulative damage from successive infusions could be lessened. Exactly, how this can be achieved remains to be seen. However, considering that most deficits occurred in the first 2 ml of infusion and the longer a deficit persists the more likely it is to be permanent, a slower ramping regime with a more responsive approach to onset of symptoms may help to reduce early deficits treatment-related morbidity.

Although these data are able to describe the pattern of deficits occurring during pontine infusion, the cause (or causes) remain unknown. There are three obvious factors to consider when exploring the sources of treatment-related morbidity; the underlying brain, the physiological effect of the infusion and the pharmacological

toxicity of the drug. Originally, the drug has been a prime suspect as the cause of morbidity. Carboplatin is neurotoxic, which is thought to be brought about by a combination of nuclear and mitochondrial DNA damage, action on ion channels and microglia cells (Kanat, Ertas and Caner 2017, Avan et al. 2015). While, on the other hand, sodium valproate can result in encephalopathy and parkinsonism (Jamora et al. 2007, Gerstner et al. 2006). The drugs in solution also possess high osmolality. Injection of hyperosmolar solutions into the ventricles causes fluid shifts between different fluid compartments within the central nervous system (Krishnamurthy et al. 2009). Abnormal fluid shifts due to intra-tumoural drug administration could therefore result in critical increase in intra-pontine pressure. The pharmacodynamic characteristics of sodium valproate and carboplatin together with the hyperosmolarity of the drugs' excipients could cause significant neuro-toxicity.

However, there is evidence to suggest there may be other factors responsible. The combination of sodium valproate and carboplatin has not been found to be directly neuro-toxic in preclinical models (Killick-Cole et al. 2017). The onset of deficit is rapid during infusion, with 30% of side-effects occurring within the first millilitre of infusion, possibly too soon for any carboplatin-mediated DNA damage or inflammation to take place. Carboplatin resides in the brain for at least 24 hours when delivered by CED (White et al., 2012). There was evidence of recovery immediately after infusion even though local drug concentration would still have been high. Also, it appears that the way the infusion is conducted seems to be associated with risk of deficits becoming persistent. Notably, neurotoxicity is often a delayed complication of carboplatin treatment and there was no clear evidence of toxicity arising after the infusion had finished suggesting that delayed carboplatin-mediated toxicity had not taken place.

Furthermore, the side-effect profiles of cranial neuropathy, ataxia and limb weaknesses were similar using carboplatin alone, sodium valproate alone, in combination and at different respective concentrations (Singleton et al. 2016; see Chapter 2 and 4). Indeed, the side-effects described by Souedaine et al., were also very similar (Souweidane et al. 2018b) despite using a totally different infusate. Patients receiving sodium valproate by monotherapy had a simultaneous four-catheter infusion, which was associated with the significant neurological deficits that failed to recover while combined therapy performed using a two-catheter infusion was only associated with mild-moderate deficits. The maximum rate of infusion occurring during a 4 catheter infusion and a 2-catheter infusion is 1.2 ml/hr and 0.8 ml/hr respectively. Taken together, this would suggest that the infusion itself is an important cause of the observed toxicity.

So what could it be about the infusion that gives rise to such neurological side effects? Previously, it was thought that the anatomical position of the catheters was a key determinant of the side-effect profile. Indeed, the first patient to receive sodium valproate by pontine infusion developed immediate facial palsy, which was improved when a catheter adjacent to the facial colliculus was retracted (see Chapter 2). Indeed, onset of unilateral weakness is often improved by reducing flow into the contralateral pons possibly due to relief of pressure within the associated corticospinal tract. However, other relationships between anatomical location of catheters and observed deficits are more difficult to explain. For example, not all patients develop the same neurological symptoms despite catheters being in broadly comparable brain regions. This could be due to several reasons. Multiple catheters still infuse concurrently preventing accurate localisation of a deficit to a specific

catheter. The tangential trajectory of catheters across the midline could also prevent lateralisation of deficits. The complex anatomy and decussation of neural structures within the pons and their distortion by the underlying tumour may also make localisation challenging. Different neural structures may also recover at different rates. After reduction of flow rates some deficits may not immediately improve, which could make interpretation of cause and effect more difficult. Neural damage due to the tumour is also not uniform and abnormal tumour architecture could result in heterogeneous fluid distribution. Beyond this complexity, a key principle of caring for the neurologically injured patient is the Munroe-Kelly Doctrine, which stipulates that the skull is a fixed vault, containing blood, brain and cerebrospinal fluid. Their volumes exist in an equilibrium where increase in one would result in '*water or other matter effused or secreted… out of the cranium*" (Munroe 1783). Indeed, this equilibrium is responsible for maintaining safe intracranial pressure (ICP), which in turn determines the pressure gradient across the brain capillary bed, known as the cerebral perfusion pressure (CPP). CPP is determined by:

$$CPP = mean\ arterial\ pressure - ICP$$

During pontine infusion, there is evidence of raised ICP. Patients complain of headache and have reported vomiting. Given that the level of consciousness is rarely impaired during pontine infusion, it is unlikely that global CPP becomes critically low. But, generating high local pressure by continuous infusion is still the core principle of CED and, therefore, in these children intra-pontine pressure is still likely to be high (Bobo et al. 1994). A possible cause of the observed neurological toxicity could be the development of localised perfusion deficits within the pons.

Considering that perfusion in DIPG varies between patients and within tumours (Sedlacik et al. 2013); this may explain why some neurological signs and symptoms are not easily localised to certain catheters and also vary between patients. Indeed, the time dependent features of infusion-related deficits are also reminiscent of Stroke Medicine's '*time is brain'*. Prompt relief of pressure by stopping infusion or reducing flow rate into critically ischaemic parts of the pons may allow adequate reperfusion and prevent ongoing injury.

Regardless of the underlying cause of these deficits, many of these hypotheses can only be tested in human subjects. As described, the neurological phenotype arising during pontine infusion is likely a product of the interaction between the location of the catheters, the pattern of fluid distribution and pathology of the underlying tumour. This requires a reliable means of quantifying neurological change, which would be hard to achieve in an animal model. Consequently, as well as developing novel clinical tools to monitor pontine infusion, another important step in the translation of CED for DIPG is the need to develop imaging techniques to quantify events at a tissue level.

In summary, this chapter presents findings from 55 pontine infusions in 8 children with DIPG. Analysis of neurological signs and symptoms occurring during pontine infusion using the PINEScore demonstrated that risk of persistent deficits were higher if the infusion was not promptly adjusted and if the deficit had occurred during a prior infusion. If catheter flow was adjusted based on anatomical position some deficits would recover. This suggests that the neurological damage arising from pontine infusion can be localised, it is time dependent and accumulative.

Development of imaging techniques will enable better understanding of the aetiology of these deficits and may address whether the underlying process is ischaemic.

Table 5.1. Transient and persistent neurological deficits acquired during 55 pontine infusion in 8 children with DIPG

	Number of transient deficits	Number of persistent deficits	Number of deficits present at 4-weeks follow-up
Reduced level of consciousness	1	0	0
Ophthalmoplegia	5	8	4
Facial dysesthesia	0	0	0
Facial weakness	12	6	3
Tongue weakness	2	2	0
Dysarthria	12	5	1
Body dysesthesia	1	0	0
Limb weakness	25	24	8
Limb ataxia	16	5	2

Table 5.2. Frequency of transient versus persistent neurological deficits according to duration of deficit, maximum severity, action taken, incidence of deficit, infusion location and volume at onset

	Number of transient deficits	Number of persistent deficits	Ratio Persistent : Transient
How long did the infusion continue after deficit onset?			
0 hours	13	6	0.46
1 hour	11	4	0.36
2 hours	13	8	0.62
3 hours	6	6	1.00
≥4 hours	31	26	0.84
What was the maximum severity of the deficit measured by the PINEScore*?			
1 point	49	27	0.55
≥2 points	23	23	1.00
What action was taken at onset of deficit?			
Infusion was continued	46	40	0.87
Infusion was titrated immediately	17	7	0.41
Infusion was stopped immediately	11	3	0.27
How many times has the deficit occurred during a previous infusion?			
Never	37	15	0.41
Once	15	14	0.93
Twice	10	9	0.90
Three or more times	11	12	1.09
Did the deficit occur during a trans-frontal infusion or a trans-cerebellar infusion?			
Trans-frontal infusion	45	22	0.48
Trans-cerebellar infusion	27	28	1.03
At what volume of infusion did the deficit occur?			
≤1 mL	24	14	0.54
1-2 mL	13	13	1.00
2-3 mL	12	14	1.17
3-4 mL	14	5	0.36
4-5 mL	11	4	0.36
*PINEScore- Pontine Infusion Neurological Evaluation Score			

Chapter 6. Development of quantitative MRI techniques to evaluate patients with diffuse intrinsic pontine glioma during pontine infusion

Background

Treating children on compassionate grounds with carboplatin and sodium valproate delivered by pontine infusion has been associated with better than expected survival in patients with diffuse intrinsic pontine glioma. However, this new treatment was also associated with neurological morbidity. The Pontine Infusion Neurological Evaluation (PINE) Score was used as a measure of neurological deterioration during infusion and demonstrated that much of this observed neurological morbidity is likely to be infusion-related rather than due to the toxicity of the drug. The cause of such infusion-related toxicity is unknown.

Exploring this question is limited by the lack of an appropriate animal model. The major model for chronic intermittent convection enhanced delivery uses pigs (Bienemann et al. 2012). Pigs share much structural homology with the human brain; they are gyrated (gyrencephalic) as opposed to smooth (lissencephalic) and the porcine brainstem has been described in detail (Hofman 1985, Freund 1969). However, the characteristics of structurally normal brain is likely to differ significantly from tumour, which makes healthy pigs a poor model to understand the deterioration observed in children with DIPG. Animal models of DIPG use mice and rats (Misuraca, 2015). But, rodent models of CED rely on one-off or repeated cannulation of the brain (Arshad et al., 2015, Bienemann et al., 2012). This creates a tract that may allow drug to reflux and pressure to dissipate, mitigating possible mechanisms contributing to infusion-related toxicity. A disease model in a large

animal would be helpful. Indeed, brainstem glioma is reported in dogs (Mateo et al. 2013, Petrukovich and Kilburn 2015, Cervera et al. 2011), but their treatment with chronic intermittent CED in an experimental setting is limited. Consequently, the best subjects to understand the basis of infusion-related toxicity in children with DIPG may be the patients themselves. As such, peri-infusion imaging presents a valuable opportunity to understand the basis of infusion-related toxicity during CED at the tissue level.

Patients receiving CED routinely undergo magnetic resonance imaging (MRI) before and after infusion (Barua et al. 2013, Barua et al. 2016, Singleton et al. 2016). MRI has been used to describe a range of phenomena in drug delivery including quantifying tumoural response and local drug concentrations. However, analysis has focused on quantification of drug distribution, which is used as a metric of catheter efficiency (Mardor et al. 2001) and an factor of therapeutic efficacy (Mueller et al. 2011). Hence, this has been the major emphasis of previous literature on the topic (Bernal et al. 2014, Chittiboina et al. 2014, Dai et al. 2016, Dickinson et al. 2008, Kim et al. 2009, Krauze et al. 2005, Lonser et al. 2002, Magdoom et al. 2014). This chapter aims to describe how MRI could be used to understand infusion-related toxicity.

MRI uses a combination of a strong magnetic fields and radiofrequency to determine different densities of protons within body tissues. T1 and T2 weighted MRI can be used to describe water molecules in terms of their concentration, their orientation and physiochemical environment (Mathur-De Vre 1984). Increased T2 signal MRI can identify increased extracellular water during CED (Sampson et al. 2007).

However, in other disease states MRI can also be used to identify intracellular water accumulation. Diffusion weighted imaging (DWI) measures the Brownian motion of water molecules within in a voxel of tissue. Changes in the DWI signals can be used in the diagnosis and treatment of various neurological diseases:

- Early diagnosis of ischaemic stroke and differentiation of acute stroke from chronic stroke
- Differentiating epidermoid from arachnoid cysts
- Differentiation of abscesses from infective cysts
- Assessment of Creutzfeld-Jacob Disease
- Differentiation of diffuse temporal glioma from herpes simplex
- Grading of intrinsic and extrinsic brain tumours
- Assessment of active demyelination

DWI signal can be quantified in many ways. However, apparent diffusion co-efficient (ADC) remains the most commonly used in clinical practice. ADC measures diffusivity and values decrease in disease states where Brownian motion of water is reduced. This can be used to identify cytotoxic oedema arising from stroke. A possible cause of infusion-related deficits may arise from localised brainstem ischaemia leading to stroke. During stroke there is a metabolic failure brought about by hypoxia. This results in failure of membrane adenosine triphosphate pumps resulting in intracellular water accumulation. This chapter explores how changes in ADC could be used to quantify these events taking place during infusion.

Methods

MRI before and after pontine infusion was included in the standard operating procedure of pontine infusion. Children with DIPG receiving pontine infusion with carboplatin and valproate as monotherapies or in combination underwent imaging acquired on a 1.5 Tesla Seimens Aera as tolerated. In addition to T1-weighted and T2-weighted images, DWI imaging were acquired at 3 b-values (0, 500, 1000) consistent with protocols designed to identify ischaemic brain (Shen et al. 2011).

MRI post-processing was performed using FSL for brain extraction and registration (Smith et al., 2014). Pre-infusion MRI was then floated onto post-infusion MRI using three-dimensional rigid registration. In order to compare changes in ADC in the same anatomical space, a cube (18 voxels^3) was selected as a region of interest using ITK Snap®. The cube was located on pre-infusion MRI by centring the cube at the intersection of two lines drawn from the lateral floor of the fourth ventricle to the contralateral temporal uncus on each side (Figure 6.1). MatLab® was used to measure absolute mean ADC values for each region of interest both pre- and post-infusion. Differences between mean ADC values were calculated by subtracting the mean ADC of the post-infusion MRI from the pre-infusion MRI on a voxel-voxel basis. Statistical significance was measured using a Wilcoxon Signed Rank Test and Chi Square Test. Statistical significance was defined by a p-value<0.01.

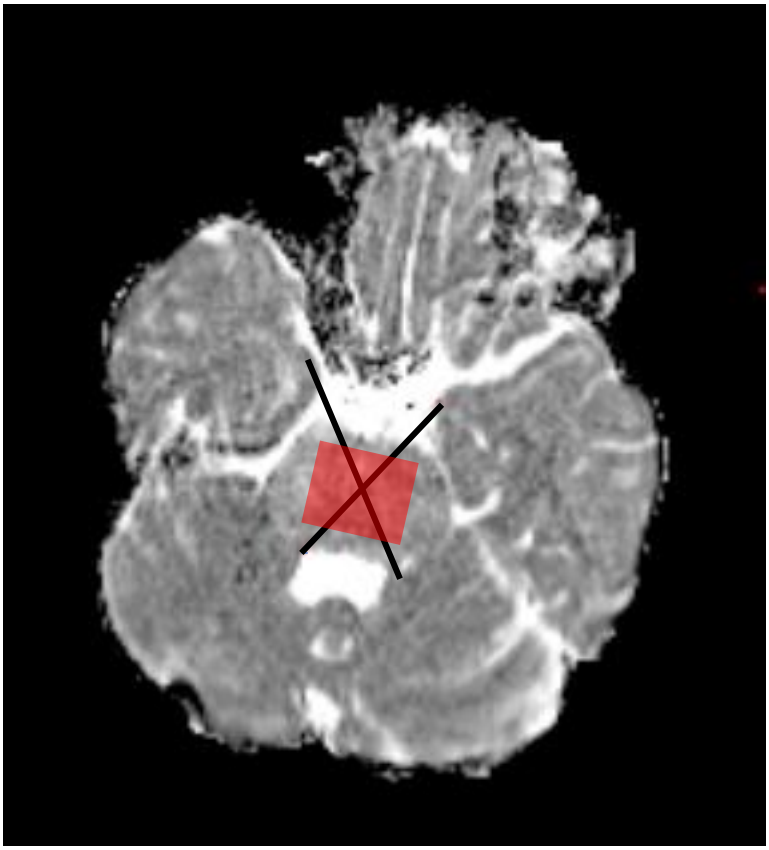

Figure 6.1. The Pontine Cube: Reference space. Analysis was conducted within an 18 voxel cube centred on the intersection between two lines drawn between the lateral floor of the 4th ventricle to the medial border of the contralateral uncus.

Results

5 infusions in 3 children had both a pre-infusion and a scan performed immediately after infusion scan sequences for T2*, fluid attenuated inversion recovery (FLAIR) and diffusion weighted imaging (DWI). Four infusions had post-infusion scans deferred to the following day. One post infusion scan was delayed until after the second infusion. The remaining infusions either did not have post-infusion imaging performed or available DWI sequences. Reasons for scans being delayed were due to infusions continuing beyond working hours where a MRI radiographer was no longer available. Scans after infusions were also not performed if they would not tolerate the MRI without a general anaesthetic.

Overall, mean ADC values increased after pontine infusion from 0.831 m/s to 0.840 m/s, which did not reach statistical significance (p-value=0.182; Table 6.1). However, this observation was not consistent amongst all 3 patients. 2 patients demonstrated right-ward shift in ADC values and one patient demonstrated no change in distribution of ADC values after infusion (Figure 6.2). The number of voxels with ADC values <0.6m/s, a value consistent with ischaemia, were calculated before and after infusion. The total number of voxels <0.6m/s before and after infusion were 434 and 277 respectively, which reached statistical significance (p-value=<0.001) (Table 6.1). Again, this reduction in the number of voxels with ADC <0.6 m/s was not consistent across all infusions. Three infusions were associated with a increase in the number of voxels <0.6m/s, all of which reached statistical significance (Table 6.1)

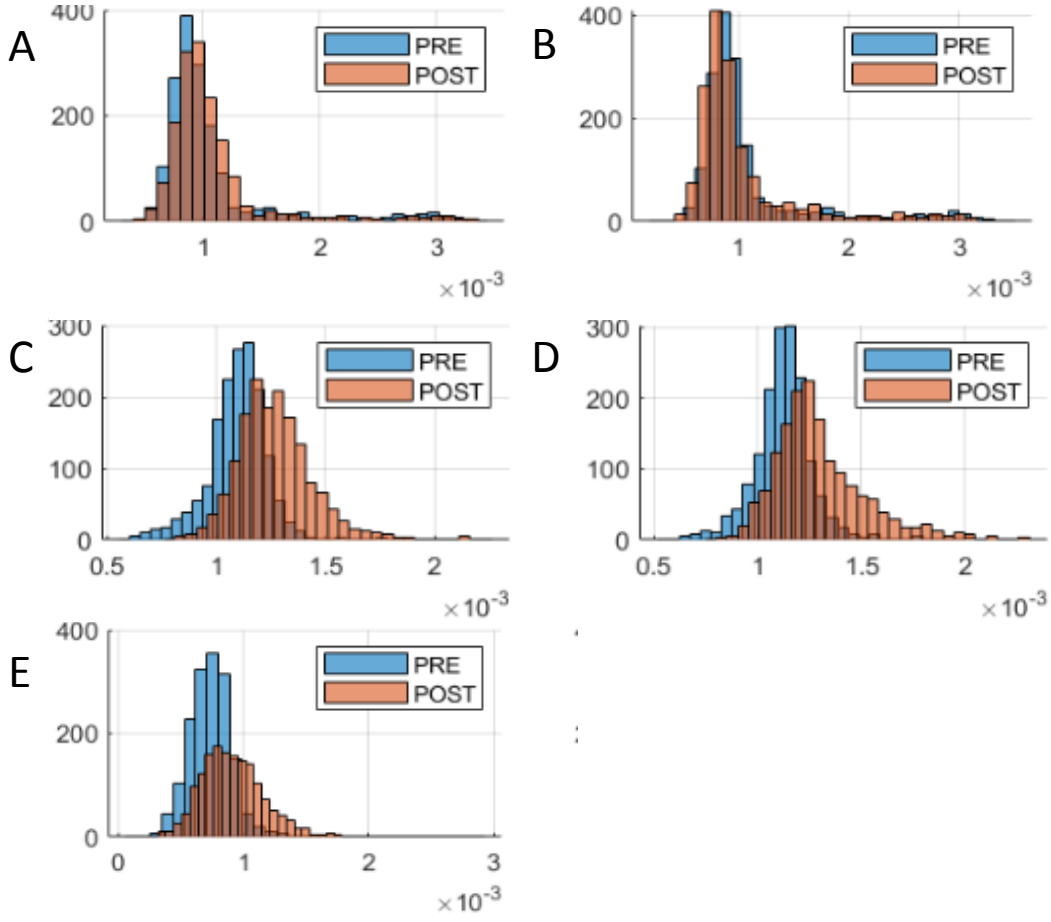

Figure 6.2. Histograms of apparent diffusion co-efficient values pre- and post-infusion in 5 infusions in 3 children with diffuse intrinsic pontine glioma. Histograms are taken from cycle 3 and 4 of patient D (A and B), cycle 4 and 5 of Patient J (C and D) and cycle 2 of Patient L (E).

The tumour microenvironment is heterogeneous and although mean diffusivity may be higher following infusion there still may be localised perfusion deficits arising from a combination of the anatomical arrangement of the catheters, asymmetric drug distribution, the vascular network of the tumour and the complex anatomy of the underlying pons. As such, diffusivity was compared before and after infusion within the region of interest voxel by voxel (Figure 6.3). This demonstrated that ADC values both increased and decreased during infusion depending on location within the pons (Figure 6.3). More voxels increased than decreased at 4086 and 4014 following infusion respectively, but this did not reach statistical significance (p-value=0.264) (Table 6.1). This suggests that changes in diffusivity occurring during infusion are heterogeneous within regions of the pons.

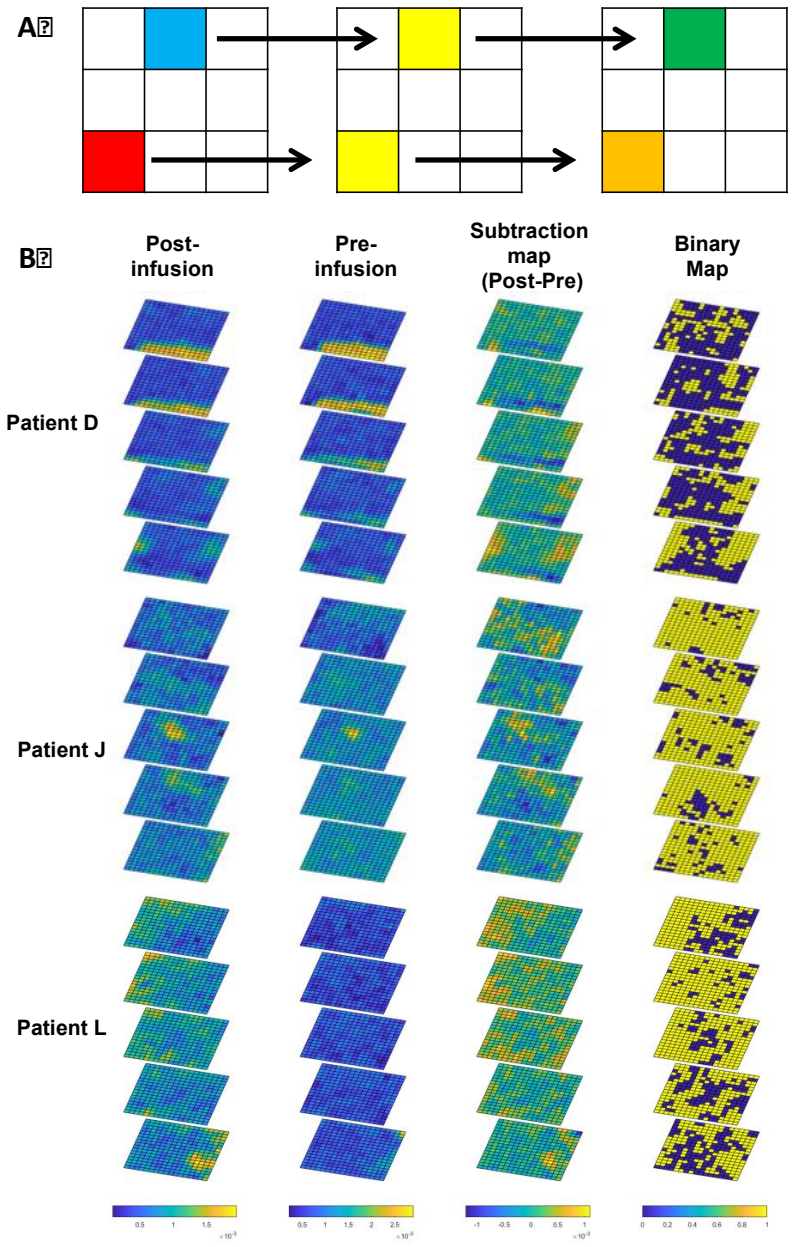

Figure 6.3. Anatomical heterogeneity of apparent diffusion co-efficient (ADC) shift in 3 children with diffuse intrinsic pontine glioma. Post-infusion images were registered to pre-infusion images. The reference space was divided into voxels. Post infusion voxel values were subtracted from pre-infusion voxel values in an anatomically specific manner (A). Representative maps from 3 patients are shown showing post-infusion and pre-infusion ADC maps: lower ADC values are indicated by darker colours. Subtraction Maps were generated by subtracting pre-infusion voxels from post-infusion voxels. Binary Maps demonstrate voxels that increased diffusivity (yellow) and decreased diffusivity (blue)

Discussion

This exploratory analysis aimed to develop quantitative fully automated MRI techniques to describe the physiological processes taking place during pontine infusion. The results demonstrate that ADC following infusion results in heterogeneous changes both between patients and within tumour. Using ADC mapping, there was no evidence that ischaemia was taking place during infusion.

There are several limitations to this imaging study. A small number of patients were used and different volumes of infusate were administered on each occasion. The contribution of tumour characteristics to changes in diffusivity is also unclear. Notably, one patient demonstrated very little change in mean diffusivity while other patients demonstrated increase in mean diffusivity. The extent to which this reflects individual particularities of drug distribution, cellular architecture of the underlying tumour or efficiency of drug delivery remains to be seen. This limited analysis would be benefitted by volumetric analysis and quantitative assessment of accompanying T2* and T1 signal intensity. The absence of follow-up imaging would also be useful to delineate the underlying physiological processes taking place. In stroke, for example, DWI undergoes a stereotypical transformation relative to the age of infarct.

Despite the limitations of this study, it was evident that pontine infusion in some cases increased diffusivity. This likely represents an increase in free water molecules arising from direct intra-parenchymal infusion. Use of DWI has been used previously to estimate volume of distribution in experimental models. Iyer et al., 2011, infused non-human primates with radiolabled dextrose and sucrose demonstrating that the volume of distribution using T2, DWI and autoradiographic studies were co-correlated. Clinically, T2-weighted imaging has been used to estimate drug distribution (Barua et al., 2013; Barua et al., 2016). This is despite evidence that T2-weighted imaging cannot demonstrate drug distribution in oedematous brain and the boundaries between infused and uninfused brain are dependent on user-defined intensity thresholds (Sampson et al., 2007). Such methods may be vulnerable to bias and may inadequately evaluate infusion efficacy and catheter efficiency. Considering that infusion volumes are maximised to increase the volume of distribution, which in

turn is associated with ongoing risk to the patient, an objective method of quantifying drug distribution is essential. The method described herein does not help delineate the boundary between infused and un-infused brain but it does use a fully automated quantitative approach to quantify drug delivery in abnormal brain, which could be developed further.

The use of MRI in CED at present is still confounded by many important factors. It may be that quantifying volume of distribution using MRI will never truly represent drug distribution at a molecular level. Iyer et al., demonstrated that volume of distribution using T2, DWI and radiolabelling co-correlated, but MRI quantification underestimated the volume of distribution compared to radioanalysis. Such error could be overcome by co-infusion of tracers such as gadolinium, which can accurately model the volume of drug distribution (Mehta et al., 2011). But, such a technique assumes that the drug and tracer distribute equally through tissues and maintain the same tissue half-life. Indeed, more recently gadolinium has been shown to deposit in the brain, the clinical significance of this is unknown (Guo et al, 2018). Considering the neurological morbidity associated with pontine infusion, delivery of a therapeutically inert substance at the risk of neurological compromise raises an ethical dilemma. It is possible to bind drug to tracer molecules to overcome these limitations (reviewed in Mehta et al., 2011). However, this could alter the therapeutic efficacy and toxicity of the drug. Given these limitations, the complexity of modelling drug distribution may require a more pragmatic method of assessing drug delivery and therapeutic efficacy. Changes in MR Spectroscopy, for example, have been used to quantify local tumour response and subsequently infer efficacy of drug delivery in CED (Guisado et al., 2016). Another approach could be monitoring of disease response within a reference space where there is objective evidence of drug delivery. Longitudinal measurement of contrast enhancement, DWI and T2 weighted signal intensity can indicate tumour progression and response (Elllingson et al., 2017, Chang et al., 2017, Radbruch et al., 2012). Tracking these characteristics this within the central pontine cube described herein may help better understand the association between drug delivery and treatment effect.

It must also be acknowledged that increase in diffusivity during infusion may be due to other factors than drug distribution. Tumours are hypercellular and restrict the

movement of water molecules with their cell membranes, macromolecules and irregular extracellular spaces; this reduces ADC. When tumours are treated with anticancer therapy there is resultant necrosis, apoptosis and cell lysis leading to increased mobility of water within tissues, which should increase ADC (reviewed in Afaq et al., 2010). It is possible therefore that a contributor to rightward shift in ADC may represent tissue lysis. Indeed, vacuolation at the end of catheter has been observed in patients with CED (Figure 6.4). We may be able to use DWI to determine critical levels of tissue tolerance and reduce structural damage resulting from infusion.

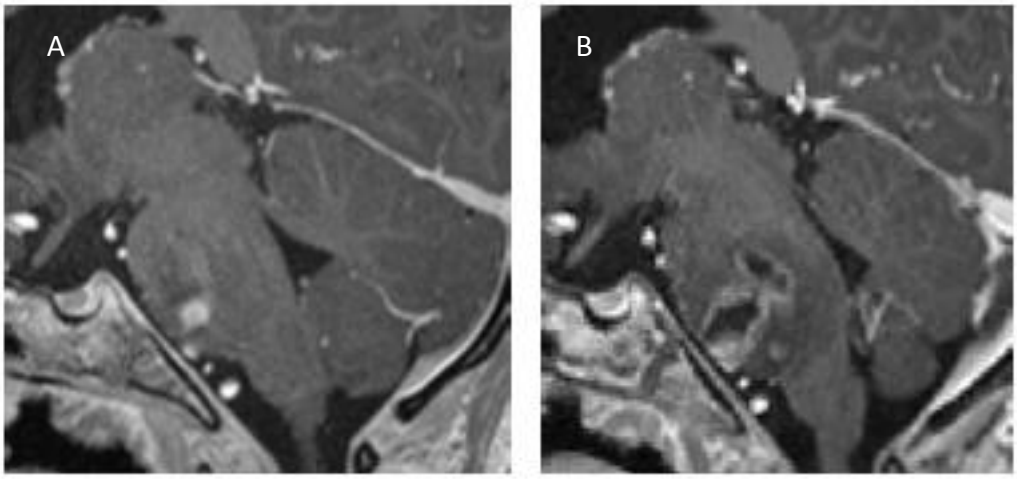

Figure 6.4. Vacuolation along a catheter trajectory following pontine infusion. Mid-sagittal plane of T1-weighted magnetic resonance image sequence with contrast before pontine infusion (A) and after pontine infusion (B) in a patient who received carboplatin monotherapy.

This chapter fails to answer if neurological deterioration in pontine infusion is due to an underlying ischaemic process. Net increase in diffusivity after CED may not necessarily mean ischaemia is absent at a cellular level. In acute stroke, DWI indicates ischaemia by reduction in ADC within a brain volume reflecting pathological cytotoxic oedema (Schaefer et al., 2000). Signal from increased extracellular free water or tissue lysis arising during CED may overwhelm any change in ADC arising from a simultaneous ischaemic process. Other methods to identify ischaemia may help to identify underlying ischaemia, such as measurement of cerebral blood flow (CBF). CBF can be measured by dynamic susceptibility contrast (DSC) MRI, using exogenous contrast, or by arterial spin-labeling (ASL) MRI that magnetically tags water molecules in blood (reviewed in Muir et al., 2014). Both techniques have been

used to diagnose stroke (Copen et al., 2012). However, evaluating intra-tumoural ischaemia requires validation in animal models and could be complicated by the already hypoxic microenvironment of DIPG (Yeom et al., 2015). Nevertheless, such techniques may be useful in delineating the cause of neurological deterioration during pontine infusion and in time help to prevent long-term disability.

In summary, this chapter demonstrates the development of a fully automated quantitative method of evaluating pontine infusion using MRI. Increase in diffusion after CED likely represents local drug delivery; however, this also could represent other processes such as tumour lysis. It does not show that neurological deterioration during infusion is due to ischaemia but suggests that other MRI modalities may be required to reject this hypothesis. Nevertheless, CED is a developing field. Advances in drug delivery system design need to be matched by advances in methods to evaluate their effect on the patient and the underlying brain. This fully automated method of image acquisition, registration and quantitative analysis could provide important insight into the physiological processes taking place during direct intra-parenchymal infusion.

Table 6.1. Quantitative Apparent Diffusion Co-efficient (ADC) data before and after 5 pontine infusions in 3 children with diffuse intrinsic pontine glioma

Patient	Infusion number	Volume infused	Mean ADC pre-infusion m/s (S.D)	Mean ADC post-infusion m/s (S.D)	p-value	Number of voxels with ADC <0.6 m/s pre-infusion	Number of voxels with ADC <0.6 m/s post-infusion	p-value	Number of voxels with increased ADC during infusion	Number of voxels with decreased ADC during infusion	p-value
J	3	4.3	0.867 (0.131)	0.842 (0.146)	<0.001*	2	14	0.005*	649	971	<0.001*
	4	4.5	0.880 (0.176)	0.775 (0.202)	<0.001*	9	184	<0.001*	381	1239	<0.001*
D	4	3.6	0.851 (0.144)	0.859 (0.155)	0.053	4	24	<0.001*	855	765	0.002*
	5	4.8	0.772 (0.225)	0.859 (0.158)	<0.001*	293	8	<0.001*	958	662	<0.001*
L	2	4.5	0.785 (0.195)	0.866 (0.188)	<0.001*	126	47	<0.001*	1243	377	<0.001*
Overall			0.831 (0.183)	0.840 (0.174)	0.183	434	227	<0.001*	4086	4014	0.264

ADC- Apparent Diffusion Coefficient; S.D – Standard Deviation; *statistically significant (p-value<0.01)

Chapter 7. Reflections on the CED-DIPG Program

This thesis describes the treatment of 15 children who underwent pontine infusion of chemotherapeutic agents by convection enhanced delivery (CED) for the treatment of diffuse intrinsic pontine glioma (DIPG) on compassionate grounds. The DIPG-CED treatment programme, which uses an experimental chronic, implantable drug delivery system, began after a small number of children DIPG were treated with carboplatin monotherapy (Singleton et al., 2016). There were encouraging signs of efficacy with children surviving longer than the expected median survival of 11. 1 months (Veldhuijzen van Zanten et al., 2017). However, the treatment was associated with neurological side-effects. These were thought to occur after infusion, and their aetiology was attributed to the pharmacological toxicity of drug, which is a well established problem in modern oncological practice.

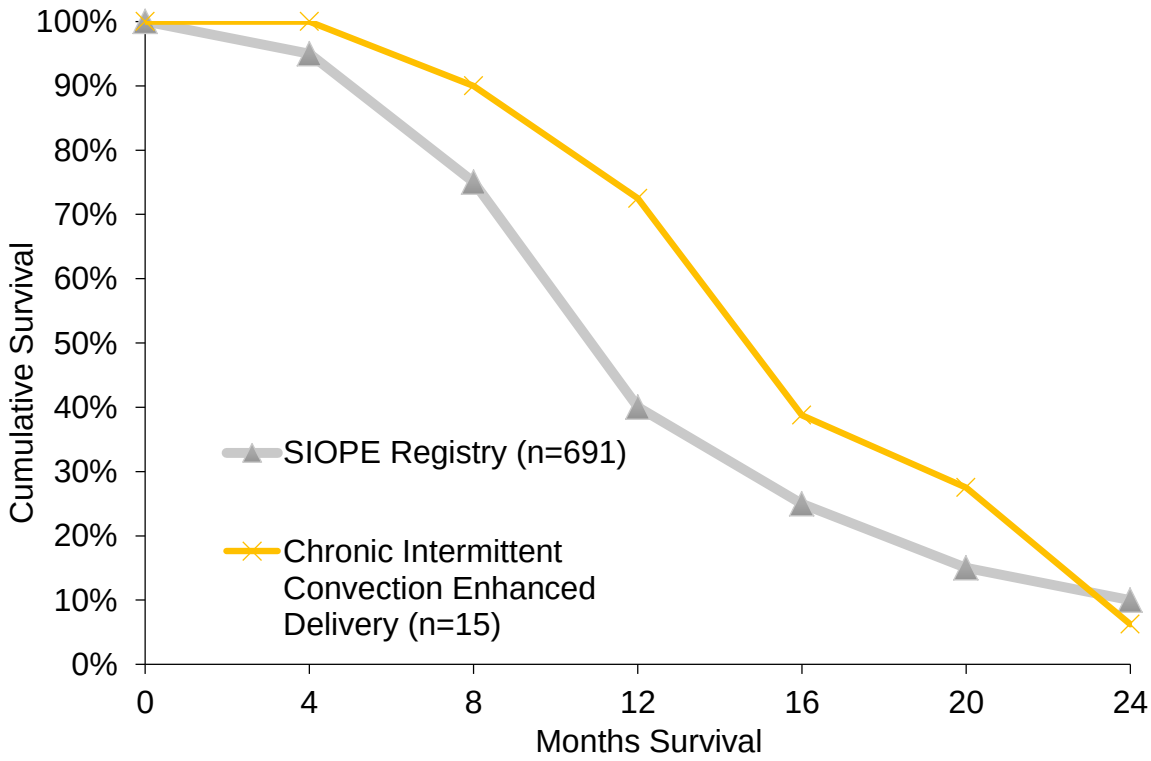

Figure 7.1. Survival curves for 15 children with diffuse intrinsic pontine glioma (DIPG) treated with chronic intermittent convection enhanced delivery on compassionate grounds versus the SIOPE DIPG Registry (Veldhuijzen van Zanten et al., 2017).

Patients in the DIPG-CED programme went onto receive, a histone deacetylase inhibitor, sodium valproate, which is where this thesis begins. Sodium valproate was intended to functionally target the histone mutations that characterise DIPG (Killick-Cole et al., 2017). As a widely used anti-epileptic drug it was expected that sodium valproate would be better tolerated when delivered by CED compared to carboplatin. However, like carboplatin, treatment was associated with neurological side-effects but whether the toxicity arose from the pharmacological effect of the drug or the effect or the infusion was still unclear.

DIPG is almost universally fatal, the process of dying is often rapid and debilitating - maximising quality of life of in these circumstances is paramount (Veldhuijzen van Zanten et al., 2017; Veldhuijzen van Zanten et al., 2016). The aim of this thesis was to develop methods of understanding the aetiology of toxicity arising from pontine CED, with a view to reducing the burden of treatment and helping build CED into a feasible treatment option for DIPG. I developed, implemented and evaluated a novel neurological assessment tool to quantify neurological deterioration during pontine infusion. The Pontine Infusion Neurological Evaluation (PINE) Score, as it is called, was adapted from established methods of neurological examination and was found to be excellently reliable when comparing results between examiners. For the first time it was possible to quantify accumulating neurological disability during infusion. Patients went onto receive a combination of carboplatin and sodium valproate, based on the rationale that carboplatin and sodium valproate worked synergistically *in vitro* (Killick-Cole et al., 2017). However, side-effects were still significant but the PINEScore enabled these neurological side-effects to be studied in more detail. This helped to identify risk factors for persistent deficits arising from infusion. Deficits

were more likely to be persistent if the child had sustained the deficit during a previous infusion and if the infusion was continued rather than stopped or slowed at deficit onset. Taken together, this suggests neurological injury from infusion is accumulative and possibly time dependent akin to the *'time is brain'* paradigm of Stroke Medicine (Hall & Hachinski, 1998). Deficits whilst infusing into the lateral pons through transcerebellar catheters were also associated with a higher risk of becoming persistent. Whether this represents a particular vulnerability of structures in the lateral pons or that most transcerebellar infusions were conducted on the second day of the each cycle remains to be seen. In addition, duration of deficit longer than 3 hours and more severe impairments trended toward persistent deficits.

The frequency of neurological side-effects at the time of administration, strongly suggests that the observed neurological toxicity is due to events occuring during infusion. In contrast to earlier experience treating children with carboplatin (Singleton et al., 2016), there was little evidence that neurological deficits occurred after infusion. It may be that the implementation of a systematic neurological assessment method allows intra-infusion disability to be recognised more readily. Before the implementation of the PINEScore, some deficits may have only been identified when the patient had resumed their normal activities. The cause of these infusion-related deficits is unknown.

Nevertheless, possible explanations could be broadly divided into two categories: either by rapid onset of pharmacological toxicity caused by the drug or physiological stress caused by the infusion. This thesis provides evidence to suggest that the latter should be a priority for further investigation. Firstly, many of the factors associated

with persistent deficits, i.e. action taken during infusion, site of brain infused and time to infusion adjustment relate to how the infusion is conducted. Secondly, carboplatin and sodium valproate were associated with similar toxicity profiles, both singly and in combination. Indeed, Souweidaine et al,. 2018 in their Phase I trial infusing a radioimmunotherapeutic agent by CED demonstrated similar signs and symptoms of cerebellar dysfunction, limb weakness and cranial neuropathy at one week post-infusion. The similarity in observed side-effects after infusion is in spite of the very different pharmacokinetic and pharmacodynamic characteristics between the drugs. Finally, the PINE score showed signs of immediate clinical improvement following infusion adjustment eventhough local tissue concentrations would still have been high. The exact physiological processes underpinning neurological deficits is unknown and should be considered in further preclinical experiments. This thesis puts forward the idea that they could be related to ischaemia. Development of a quantative MRI protocol to address this hypothesis was unable to exclude or confirm the presence of ischaemia during pontine infusion, but could form the basis for further analysis.

There are many positive findings that show great promise for the treatment. Patient's survived longer than expected for the wider DIPG population. Patient A demonstrated disease response following treatment with sodium valproate. Patient D and J progressed outside the volume of infusion suggesting that CED was able to achieve local control of disease. We were able to achieve repeated pontine infusions in 14 of the patients implanted with the device. Evidence that side-effects of CED are due to infusion means the treatment burden could be improved by adjustment of infusion parameters. Infusion-related deficits were not volume dependent, i.e. deficits

acquired at greater than 3 mL volume of infusion were paradoxically less likely to be persistent. Pontine infusion could potentially be performed long term and achieve even greater volumes of infusion providing asymtpomatic infusions can be achieved. Further to this, no systemic toxicity was identified, which could pave the way for children with brain tumours to receive anatomically targetted therapy whilst avoiding the side-effects of systemic chemotherapy.

These conclusions are made, however, with several important caveats. Patients survived longer than expected compared to the wider DIPG population. The children implanted with the drug delivery system had a median suvival of 13. 9 months, with 5 children surviving over 20 months. Although, this is encouraging, it must be acknoweldged that these patients represent an extraordinary sample of children. Their families have sort experimental treatment, mostly in a country foreign to their own and at significant personal expense. They were also selected on the basis of radiological and clinical factors that made implantation of the drug delivery system feasible at a time when a proportion of patients would already have had advanced disease or died. The extent to which these biological and sociological factors impacted survival remain to be seen. Moreover, many patients went onto receive additional experimental therapies and the contribution of this to the observed survival is unknown. As such, the extent to which results from these patients can be extrapolated to the treat DIPG as a whole is limited and needs to be interrogated in a controlled trial. Experience from these children demonstrates that this treatment is feasible, and if combined with an appropriate method of clinical monitoring as described in this thesis, it could be delivered safely. Conclusions regarding the risk factors for persisient infusion-related deficits are limited by how the PINEScore has

been implemented and validated, which again should also be further explored in a clinical trial. The robustness of the PINEScore would be improved by assessment of intra-rater reliability; this would provide reassurance that the PINEScore could assess recovery and deterioration *between* as well as *during* infusions. The PINEScore was validated in children in various stages of middle childhood and early adolescence and data was derived from a very small number of children. Patients had different nationalities and different first langauges. PINEScore data is also collected from nursing staff with different levels of training and experience. Hence, in future the use of the PINEScore should be used after appropriate competency assessment to ensure a standard quality of examination. In addition, diagnosis of transient and persistent deficits, on which much of this analysis relies, would be better recorded by an independent investigator blinded to the treatment received.

Depite these limitations, treating patients on compassionate grounds offered a valuable opportunity to learn how to deliver drugs safely into these tumours and have yielded some encouraging results. Such practice outside a clinical trial still raises a number of ethical issues. Compassionate use is a means of providing seriously ill patients with access to experimental treatment. Justifications for such therapy can be broadly categorised into fairness, a will to improve the patient's outcome and to facilitate autonomy of the patient (Raus, 2016).

Compassionate use programmes can provide experimental treatment to patients, who would otherwise be denied access, helping to correct a perceived injustice. After all, why should a child be denied a potentially life-saving treatment while another is able to receive it? Such circumstances could arise due to the absence of an open

clinical trial or patient's own ineligibility due to co-existing medical problems, age or stage of disease. Offering dying patients who have no other treatment options experimental treatment is also intended to improve their outcomes. The will to offer treatment out of beneficence is an important and common justification for giving experimental treatment. Finally, out of respect for the patients autonomy, if dying patients *"freely take on the risks of an experimental drug for the chance of great benefit, who are we to refuse them?" (Raus 2016).*

Such arguments are emotive and have lead to significant media and political exposure of patients being denied access to innovative therapies. There are even examples from patients with DIPG seeking CED (Blott, 2018). High profile Labour MP, Tessa Jowell, died from glioblastoma multiforme in 2018, her campaign was described by Sir Harpal Kumar, Chief Executive of Cancer Research UK, as '*a call for more research and innovative treatments to be made available'* (Drewett, 2018). Such attention emphasizes the urgency to improve outcomes for patients with little chance of cure. On a wider scale, these arguments have resulted in many US States to employ *'Right-to-Try'* laws, which grant seriously ill patients access to unapproved treatments (Zettler et al., 2014; Bateman-House et al., 2015). Social media and online petitions lobby governments and the medical industry to loosen regulatory constraints on new treatments (Mackey & Schoenfield 2016). While websites such as www.mytomorrows.com profit by providing access to unapproved treatment (Raus et al., 2016).

Although, there is a social appetite for such practices, experimental treatment outside of a clinical trial can be problematic. Compassionate treatment is sometimes

justified by providing treatment to those who have been rejected from clinical trials, which can be perceived as unfair. It could be argued that compassionate treatment could only be 'fair' if it is accessible to all. But, compassionate treatment is not necessarily allocated in a way that is consistent. Some cases are approved following significant pressure from high profile campaigns while those with less successful campaigns are not (Mackey & Schoenfield 2016). Some pharmaceutical companies have to operate a lottery system to ration experimental treatment. Indeed, if patients are charged for the care they receive there is an obvious financial limitation that would deny patients who cannot afford it.

Compassionate treatment can be offered out of a desire to help the patient. Rationally even treatments given as a part of a clinical trial, which have undergone stringent preclinical testing are unlikely to go onto gain approval. 26%, 34% and 57% of drugs tested in Phase I, II and III trials respectively go onto be approved for clinical use (Dimasi & Grabowski, 2007). Therefore, when experimental drugs are accessed outside clinical trials the chance of benefit are likely to be even smaller. It is also acknowledged that patients treated on compassionate grounds are vulnerable to exploitation, which can undermine any beneficent intention all together (Raus et al 2016). Medical industry and treating physicians can stand to make financial gain or can be a source of *'cheap and easy'* research subjects (Raus, 2016; Werthemier, 2008). Because compassionate treatment forms part of clinical practice, patients are also not protected by the same regulations used in clinical trials. There are no obligations to share data regarding efficacy or toxicity, to release generalizable conclusions by using a control group, to use peer-reviewed treatment protocols or to secure insurance to protect patients (Walker et al, 2014). It is arguable that such

risks are ameliorated when treating patients who have a palliative diagnosis. Denying patients experimental therapy is based on the precaution that the risks are unknown. Walker et al (2014) remark that this loses its *'moral force'* when the patient is already facing death. On the other hand, for patients who have a limited life expectancy, quality of survival is imperative and treatment still risks causing unnecessary suffering. When this is combined with the financial implications of such treatments on patients and their families, who compassionate treatment actually benefits can be difficult to determine (Raus et al., 2016).

However, even randomised clinical trials (RCTs), the mainstay of evidence-based medicine, are beset by the ethical implications when developing new treatment. Kodish (1991) argued that clinical trials are only justifiable if the patient can freely choose to participate. If new treatments are only available through RCTs then, as Schuklenk (2014) argues, *'dying patients are coerced into participating*'. Such coercion undermines patient autonomy and can undermine clinical trial design. Using the AIDS epidemic as an example, patients '*lied and cheated to get into trials and left in such large numbers as to threaten the viability of the AIDS clinical trial system*' (Schuklenk, 2014). It is argued that only with the availability of compassionate treatment alongside RCTs, can the patient truly assert their autonomy over using new therapies (Kodish 1991).

Compassionate treatment could be seen to empower patient choice but ensuring that the patients truly have free choice is complex. Clinical trials are subject to research governance that safeguard how patients are consented to participate. Compassionate treatment is not within a construct of a clinical trial and it is offered

as part of clinical care. There is little to protect patients overlooking the experimental nature of the treatment and they could easily assume its benefits (Raus, 2016). When a child is recognised to have no realistic chance of cure, 20% of parents who opted for chemotherapy intended to cure their child while 14% were still hoping for a cure (Mack et al, 2008). It is possible therefore that parents seeking experimental treatment for DIPG, which is terminal at diagnosis, may be particularly vulnerable and should be carefully protected.

Nevertheless, despite the ethical challenges of compassionate treatment, the outcomes for patients with DIPG are poor and have not improved despite numerous clinical trials (Hargrave et al., 2006). Ethical deliberations about the how best to improve outcomes do little to extend the survival of patients who are currently being diagnosed. Possible solutions to accelerate access to treatment whilst generating robust clinical data have been suggested, these are known as adaptive trials.

Traditionally, regulatory approval for a new treatment must pass through Phase I, II and III clinical trials: from first-in-human studies, to exploring its activity in a small number of patients, to comparing the new treatment against a placebo or conventional medical therapy. This process can take at least 7 years (Kaitlin, 2010). However, with increasing pressure to expedite progress, new methods of translating treatment from bench to bedside are being employed. In 2011, pharmaceutical company, Merck, conducted a first-in-human trial of immune check-point inhibitor, Pembralizumab, in patients with advanced solid tumours (NCT01295827). The impressive response rates prompted rapid increase in sample size, ultimately recruiting 1200 patients over the next three years. Within a short time, primary data

demonstrated that Pembralizumab extended survival of patients with metastatic melanoma, after which it became licensed for medical use. One suggestion to accelerate progress is that new treatments with unprecedented evidence of clinical efficacy should be given 'break-through status' to allow sample sizes to be expanded - upgrading the stage of trial seamlessly (Prowell et al., 2016). Other methods, as reviewed by Mahajan & Gupta, 2010, include allowing patients from early phase clinical trials to roll into later phase clinical trials, based on factors such as predictive biomarkers. 'Pick-the-winner' designs begin with several treatment arms or dosage schedules versus placebo. With time the best treatment strategy is continued, so at completion there is sufficient data to support regulatory approval for the 'winning' treatment. Another option is to allow trial hypotheses to be more responsive: allowing them to change from non-inferiority to superiority studies or switch between primary and secondary end-points. Phase II and Phase III designs can be combined whereby a study is powered for a Phase III trial on the *proviso* that it successfully completes the Phase II 'learning' stage. Patients can also be recruited to trials where patients can switch between treatment if there are concerns about safety or efficacy. This would allow more than one treatment to be tested at once. N-of-1 trials can also be conducted, which are appropriate when RCTs are unfeasible due to rarity of disease (Collette & Tombal 2015). They provide a specific way of testing efficacy of two or more treatments in a single patient. This may be appropriate when studying slowly evolving cancers, disorders where there is an early surrogate marker of efficacy or treatment that may be given repeatedly without accumulative toxicity. Consequently, although accessing new treatment through RCTs may be too cumbersome for all desperately ill patients to tolerate, there are many other clinical

study designs that have the potential to rapidly translate experimental therapies that avoid the pitfalls of compassionate treatment.

The future of CED for DIPG

So what next? Following the publication of the trial by Souweidaine., et al 2018, ^{124}I coupled to B7H3 has been extended to a Phase II trial. However, while CED treatment schedules consist of single infusions or are conducted under general anaesthetic, it is likely that treatment effect will be diminished and infusion-related toxicity will go uncontrolled. The experience of treating patients with DIPG on compassionate grounds using chronic intermittent CED shows much promise for the future. The blood brain barrier is a major obstacle for developing new treatments for patients with neurological disease. Achieving high local tissue concentrations of drug using CED could help achieve maximum local effect and minimal systemic toxicity. Particularly, this technique allows patients to be infused awake allowing infusion-related toxicity to be monitored and potentially controlled.

The limiting factor for brainstem CED is sustaining local tissue concentrations through adequate treatment volumes for long enough to exert therapeutic benefit. Prolonging infusion is limited by neuro-toxicity, because pushing infusion after the onset of deficit is unsafe. Controlling and understanding this infusion-related toxicity is a rate-limiting step in performing effective CED for DIPG. This thesis argues that the toxicity is most likely due to physiological effects of the infusion. Previously, prolonging infusion was justified in order to try and maximise the volume of treatable brain. Prolonged infusion was associated with accumulative neurological toxicity that resulted in increased recovery time, reduced treatment frequency, which overall

would have reduced tumoural drug exposure over time. Delivering infusions in such a way that patients remain asymptomatic could be achieved by reducing infusion volume targets or flow rates per catheter, being more responsive to clinical change during infusion. Also the number of implanted catheters could be increased so the same volume of distribution can be achieved with smaller pressure gradients at individual catheter tips. Minimally symptomatic infusion would allow patients to be infused with greater frequency thereby maintaining the high intra-tumoural concentrations for longer. In the future, using this method, brainstem CED could be conducted at higher intensity akin to conventional chemotherapy regimens. This could allow patients with DIPG to complete treatment within 6-8 weeks and then rehabilitate enabling them to enjoy their remaining survival unhindered by prolonged medical intervention.

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